ABSTRACTS

PLATFORM SESSION 1: ANALYTICAL I - HRMS & INNOVATIONS TUESDAY, SEPTEMBER 22 | INTERNATIONAL NORTH BALLROOM MODERATORS: LUKE RODDA & BRIGITTE DESHARNAIS			
TIME	**#**	**TITLE**	**PRESENTER**
8:00-8:10 AM	O-001	50-Year Retrospective and Appreciation: Journal of Analytical Toxicology (JAT)	Frank Peters
8:10-8:20 AM	O-002	From Method to Mainstay: One Year of LC-QTOF-MS	Maria Sarkisian
8:20-8:30 AM	O-003	Optimizing Sample Extraction and Data Review of LC-QTOF/MS All Ions Screening Using Microsoft Excel	Munchelou Gomonit
8:30-8:40 AM	O-004	Integration of an All Ions HRMS and LC Screener Strategy for Detection of Emerging and Prevalent Psychoactive Compounds	Robert Lockwood
8:40-8:50 AM	O-005	Multi-Platform GC-EI-HRMS/LC-ESI-HRMS Characterisation of Post-Mortem Vitreous Humour for Forensic Applications Using a Curated Open-Access GC-HRMS Library for GC-Based Annotation	Leen Jacobs
8:50-9:00 AM	O-006	Supercritical Fluid Chromatography Coupled to High-Resolution Mass Spectrometry: Application to the Detection of Drugs of Abuse in Oral Fluid	Franck Saint-Marcoux

PLATFORM SESSION 1: ANALYTICAL I - HRMS & INNOVATIONS TUESDAY, SEPTEMBER 22 | INTERNATIONAL NORTH BALLROOM MODERATORS: LUKE RODDA & BRIGITTE DESHARNAIS			
TIME	**#**	**TITLE**	**PRESENTER**
9:00-9:10 AM	O-007	From Research to Routine - Evaluating Endogenous Biomarker's Performance Indicating Sample Storage Conditions of Blood Specimens in 489 Authentic Forensic Toxicology Samples	Annina Bovens
9:10-9:20 AM	O-008	Native Peptide Analysis for the Detection of Sophisticated Synthetic Urine Products	Lana Brockbals
9:20-9:30 AM	O-009	Ultra-Fast Detection of Drug Cutting Agent in Urine Using LDTD-MS/MS in Under 11 Seconds per Sample	Mégane Moreau
9:30-9:40 AM	O-010	Development of an Automated Extraction and Quantitative LC-MS/MS Method for Prescription and Over-the-Counter Medications in Blood DWI Cases	Erika Pennings
9:40-9:50 AM	O-011	Ion Mobility-Enhanced Broad-Scope Forensic Toxicology Screening in Urine	Athanasia Byzyka
9:50-10:00 AM	O-012	Ion-Exchange Online-SPE for the High-Throughput Analysis of Multi-Class Drugs in Human Plasma	Jeremy Smith

PLATFORM SESSION 2: DRUGS & DRIVING I TUESDAY, SEPTEMBER 22 | INTERNATIONAL SOUTH BALLROOM MODERATORS: ANDRE SUKTA & JENNIFER LIMOGES			
TIME	**#**	**TITLE**	**PRESENTER**
8:00-8:10 AM	O-013	50-Year Retrospective and Appreciation: Journal of Analytical Toxicology (JAT)	Dayong Lee
8:10-8:20 AM	O-014	Beyond Illicit Drugs: Psychotropic Medications and DUID in France: Findings From the NPS ALERT (New Psychoactive Substances Analysis, Legal Enforcement, and Road Traffic) Study	Nadia Arbouche
8:20-8:30 AM	O-015	Comparison of Blood Alcohol Concentrations in Seriously Injured Drivers in Motor Vehicle Crashes With DUI Casework	Kristin Kahl
8:30-8:40 AM	O-016	An Evaluation of Low Benzoylecgonine Concentrations in Urine	Lauren Johann
8:40-8:50 AM	O-017	Prevalence of Ketamine in DUI cases in Colorado 2025	Tirzah Vidaurri
8:50-9:00 AM	O-018	Mitragynine Minefield – A Retrospective Analysis of Colorado Casework With Impaired Driving Case Studies	Stephanie Olofson

PLATFORM SESSION 2: DRUGS & DRIVING I TUESDAY, SEPTEMBER 22 | INTERNATIONAL SOUTH BALLROOM MODERATORS: ANDRE SUKTA & JENNIFER LIMOGES			
TIME	**#**	**TITLE**	**PRESENTER**
9:00-9:10 AM	O-019	Comprehensive LC-Orbitrap-MS Screening and Interpretation of Polydrug Exposure in Post-Mortem DUID Cases	Jose L Costa
9:10-9:20 AM	O-020	Evaluating Two Years of Impaired Driving Surveillance in Arkansas through the Arkansas State Crime Laboratory–Glen F. Baker Public Health Laboratory Collaboration	Mary Lynn Heffington
9:20-9:30 AM	O-021	Prevalence of Psychoactive Substances Among French Drivers: Key Findings From the NPS ALERT (New Psychoactive Substances Analysis, Legal Enforcement, and Road Traffic) Study	Adeline Knapp
9:30-9:40 AM	O-022	Old is Gold: Texas Breath Alcohol Testing	Liza Chacko
9:40-9:50 AM	O-023	Evidence Based Policy: The Role of Oral Fluid Testing in Drug Impaired Driving Legislation in New Zealand	Muhammad Taimoor Chaudhary
9:50-10:00 AM	O-024	Determinants of Tetrahydrocannabinol and Cocaine in Oral Fluid	Franck Saint-Marcoux
10:00-10:30 AM - MORNING BREAK			

PLATFORM SESSION 3: ANALYTICAL II - QUALITY & TROUBLESHOOTING TUESDAY, SEPTEMBER 22 | INTERNATIONAL NORTH BALLROOM MODERATORS: ALBERTO SALOMONE & MATTHEW LEVITAS			
TIME	**#**	**TITLE**	**PRESENTER**
10:30-10:40 AM	O-025	Preparation of A-Series Nerve Agent – Tyrosine Adduct Standards for Forensic LC-MS/MS Analysis	Akinori Yamaguchi
10:40-10:50 AM	O-026	Generating an Internal Standard In Situ: A Practical Isotope-Coded Derivatization Strategy for Quantitative LC–MS/MS Analysis of Urinary THC-COOH	Maiko Kusano
10:50-11:00 AM	O-027	Material Matters: Evaluating Polymer 96-Well Plates to Reduce DAMGO Loss in LC-MS/MS-Based in Vitro μ-Opioid Structure Affinity Assay	Gaia Alluisetti
11:00-11:10 AM	O-028	Overcoming Conversion, Chromatography, and Stability Challenges in the Analysis of Mitragynine-Related Compounds	Megan Dunkle
11:10-11:20 AM	O-029	Misidentification of TFMPP in Forensic Toxicology: A Cautionary Case Involving Aripiprazole Metabolism	Karolina Nowak

PLATFORM SESSION 3: ANALYTICAL II - QUALITY & TROUBLESHOOTING TUESDAY, SEPTEMBER 22 | INTERNATIONAL NORTH BALLROOM MODERATORS: ALBERTO SALOMONE & MATTHEW LEVITAS			
TIME	**#**	**TITLE**	**PRESENTER**
11:20-11:30 AM	O-030	The Meth Mimic: Unmasking N-Isopropylbenzylamine in Forensic Analysis	Rebecca Wood
11:30-11:40 AM	O-031	Characterization of Adduct Formation by Co-Eluting Compounds During LC-MS Ionization	Lea-Celine Hörcher
11:40-11:50 AM	O-032	Drift You Didn't Know You Had: Development and Validation of an Improved HS-GC-FID Method for Quantitative Ethanol Determination Across Matrices in Forensic Toxicology	Nik De Brabanter
11:50 AM-12:00 PM	O-033	Third Time's the Charm: Revalidation of an Alcohol Analysis Method	Leonela Placencia

PLATFORM SESSION 4: DRUGS & DRIVING II TUESDAY, SEPTEMBER 22 | INTERNATIONAL SOUTH BALLROOM MODERATORS: SARA DEMPSEY & AYA CHAN-HOSOKAWA			
TIME	**#**	**TITLE**	**PRESENTER**
10:30-10:40 AM	O-034	Untargeted GC-MS Metabolomic Profiling of Oral Fluid From Psychoactive Drug-Positive Drivers: Exploratory Assessment of Ketamine-Associated Metabolic Alterations	Miriam Blanco-Ces
10:40-10:50 AM	O-035	Breaking the Law, Not the Habit: A Decade of Methamphetamine Related Driving While Impaired (DWI) Casework in New Mexico	Jacob Davis
10:50-11:00 AM	O-036	On-Site Testing, LC-MS/MS and LC-HRMS in DUID Investigations: A Head-to-Head Analytical Performance Study From the French NPS ALERT (New Psychoactive Substances Analysis, Legal Enforcement, and Road Traffic) Study	Théo Willeman
11:00-11:10 AM	O-037	Three DUI Case Studies of Ketamine Only Cases in the State of Colorado	Ecem Yildiz
11:10-11:20 AM	O-038	Evaluation of the Draeger DrugCheck 3000 (DDC3000) as a Roadside Oral Fluid Screening Device Including Fentanyl Detection and Cross-Reactivity Assessment	Jasmine Maxwell

PLATFORM SESSION 4: DRUGS & DRIVING II TUESDAY, SEPTEMBER 22 | INTERNATIONAL SOUTH BALLROOM MODERATORS: SARA DEMPSEY & AYA CHAN-HOSOKAWA			
TIME	**#**	**TITLE**	**PRESENTER**
11:20-11:30 AM	O-039	A Retrospective Evaluation of Oral Fluid DUID Casework Data in New York State	Gregory Sarris
11:30-11:40 AM	O-040	Trust the Signs? A Data-Driven Look at DRE Evaluations	Amanda Mohr
11:40-11:50 AM	O-041	Gabapentin in Impaired Driving Casework in Dallas County From 2018 to 2025	Sara Dempsey
11:50 AM-12:00 PM	O-042	Prevalence of Psychoactive Drugs in Injured Drivers Over 10 Years in Victoria, Australia	Dimitri Gerostamoulos
12:00-1:30 PM - LUNCH			

PLATFORM SESSION 5: NPS I - TOXICOSURVEILLANCE TUESDAY, SEPTEMBER 22 | INTERNATIONAL NORTH BALLROOM MODERATORS: CURT HARPER & REBECCA WOOD			
TIME	**#**	**TITLE**	**PRESENTER**
1:30-1:40 PM	O-043	Strengthening Toxicological Preparedness Through the EUDA Network of Forensic and Toxicological Laboratories	Thomas Néfau
1:40-1:50 PM	O-044	Investigating the Expanding World of Commercially Available Psychotropics	Max Denn
1:50-2:00 PM	O-045	Integrated Toxico-Surveillance and Early Warning System in New South Wales, Australia: Government-Led Model With Multiagency and Community Partnerships	Thanjira Jiranantakan
2:00-2:10 PM	O-046	Lessons From the UNODC Early Warning Advisory on the Changing Face of NPS and How Systems Need to Adapt	Kathrina Hlela
2:10-2:20 PM	O-047	The Potential Displacement of 'Nitazenes' with 'Orphines' on the Novel Synthetic Opioid Market	Donna Papsun
2:20-2:30 PM	O-048	Recent Decline of Designer Opioid Positivity in Clinical Specimens	Amanda Pacana

PLATFORM SESSION 5: NPS I - TOXICOSURVEILLANCE TUESDAY, SEPTEMBER 22 | INTERNATIONAL NORTH BALLROOM MODERATORS: CURT HARPER & REBECCA WOOD			
TIME	**#**	**TITLE**	**PRESENTER**
2:30-2:40 PM	O-049	From 4-MMC to 2-MMC: Wastewater-Based Evidence of an Isomeric Shift in Methylmethcathinone Use in Belgium	Natan Van Wichelen
2:40-2:50 PM	O-050	Wastewater-Based Epidemiology as a Complementary Tool to Multimodal New Psychoactive Substance Toxicosurveillance	Jared Brown
2:50-3:00 PM	O-051	From Signal to Surveillance: A Wastewater-Based Forensic Intelligence Framework for Emerging Drug Threats in Türkiye	Merve Kuloglu Genc
3:00-3:10 PM	O-052	Wastewater-Based Epidemiology as an Early Indicator and Preparedness Tool: Rapid Changes in Synthetic Cathinone Markets in Finland	Teemu Gunnar
3:10-3:20 PM	O-053	A Tri-State Surveillance Study of Regulated and Unregulated Cannabinoid Products	Jessemia Meekins
3:20-3:30 PM	O-054	From Paper to Powders: Variability in Synthetic Cannabinoid Purity in Scottish Prisons	Victoria Marland

PLATFORM SESSION 6: ALTERNATIVE MATRICES I TUESDAY, SEPTEMBER 22 | INTERNATIONAL SOUTH BALLROOM MODERATORS: DONNA COY & LAURIE GHEDDAR			
TIME	**#**	**TITLE**	**PRESENTER**
1:30-1:40 PM	O-055	Chemometric-Driven Optimisation and Validation of Phosphatidylethanol Quantification in Dried Saliva Spots: A Green, Non-Invasive Approach for Forensic Alcohol Biomonitoring	Berfin Sude Ertas
1:40-1:50 PM	O-056	Is Anhydroecgonine Methyl Ester in Oral Fluid a Reliable Marker of Smoked Cocaine Use?	Ana de-Castro-Ríos
1:50-2:00 PM	O-057	Three-Way Comparison of Oral Fluid Transfer Methods: Evaluation of the Restek Oral Fluid Accessory, Sarstedt Salivette®, and Quantisal Alabama Department of Forensic Sciences Plunger Methodology	Jessica Jackson
2:00-2:10 PM	O-058	Development of an in vitro Model as a Basis for Characterizing Drug Accumulation in Dental Biofilms	Sabine Peisert
2:10-2:20 PM	O-059	From Drug Use to Filter: A Mechanistic Framework for Exhaled Breath Aerosol Detection of Drugs of Abuse	Christopher Suiter
2:20-2:30 PM	O-060	First Results From Breath Measurements of Acute Cannabis Elimination (BACE)	Jennifer Berry

PLATFORM SESSION 6: ALTERNATIVE MATRICES I TUESDAY, SEPTEMBER 22 | INTERNATIONAL SOUTH BALLROOM MODERATORS: DONNA COY & LAURIE GHEDDAR			
TIME	**#**	**TITLE**	**PRESENTER**
2:30-2:40 PM	O-061	Examining the Placental Transfer of Methamphetamine and Its Influence on the Human Placenta Using ex vivo Human Placenta Perfusion	Daniela Wissenbach
2:40-2:50 PM	O-062	Quantification of GLP-1 Receptor Agonists Semaglutide and Tirzepatide as Well as Insulin Variants Degludec and Icodec in Postmortem Vitreous Humor Using a Combined LC-MS/MS Method	Hans Brabec
2:50-3:00 PM	O-063	Postmortem Skeletal Toxicology: A Fresh Approach to a Persistent Problem	Christine Pink
3:00-3:10 PM	O-064	Broad-Scope Alternative Matrix Method: Development and Validation of LC–QTOF-MS Method for Detection of 946 Drugs and Metabolites in Postmortem Tissue	Jihau Yu
3:10-3:20 PM	O-065	Detecting Prenatal Alcohol Exposure Using PEth in Dried Blood Spots and LC-MSMS	Stephanie Rodriguez
3:20-3:30 PM	O-066	Comparison of POPEth, PLPEth, Ethyl Glucuronide, and Ethyl Sulfate Detection Across Biological Matrices	Marta Concheiro-Guisan
3:30-4:00 PM - AFTERNOON BREAK			

PLATFORM SESSION 7: NPS II - SYNTHETIC CANNABINOIDS & CATHINONES TUESDAY, SEPTEMBER 22 | INTERNATIONAL NORTH BALLROOM MODERATORS: ALEX KROTULSKI & MARTA CONCHEIRO-GUISAN			
TIME	**#**	**TITLE**	**PRESENTER**
4:00-4:10 PM	O-067	Pediatric Intoxication to the Synthetic Cannabinoid 5F-ADB via Vaping Devices: An Alarming Case Series	Coralie Boudin
4:10-4:20 PM	O-068	Toxicokinetic Profiling and Metabolic Characterization of MDMB-4en-PINACA in Zebrafish (Danio Rerio) Using LC-MS/MS and LC-HRMS	Leonardo Costalonga Rodrigues
4:20-4:30 PM	O-069	Pharmacological Characterization of Hexahydrocannabinol-Derived Semi-Synthetic Cannabinoids at the Cannabinoid Type 1 Receptor Using a β-Arrestin2 Recruitment Assay	Marjolein Coulier
4:30-4:40 PM	O-070	Structure-Activity Relationships of ADB-HEXINACA Analogues and Their Metabolites at the CB1 Receptor	Steven Baginski
4:40-4:50 PM	O-071	Analysis of Phytocannabinoids and Semisynthetic Cannabinoids From Postmortem Blood Samples Using Gas Chromatography-Tandem Mass Spectrometry (GC-MS/MS)	Raimo Ketola

PLATFORM SESSION 7: NPS II - SYNTHETIC CANNABINOIDS & CATHINONES TUESDAY, SEPTEMBER 22 | INTERNATIONAL NORTH BALLROOM MODERATORS: ALEX KROTULSKI & MARTA CONCHEIRO-GUISAN			
TIME	**#**	**TITLE**	**PRESENTER**
4:50-5:00 PM	O-072	Bioisosteres - In Vitro Hepatic Metabolism of Six Cathinone Related Methylthiophene Stimulants	Tilman Arnst
5:00-5:10 PM	O-073	In Vitro Characterization and Structure-Activity Relationships of Methcathinone Analogues: Insights Into Stimulant Activity and Potential Risks	Emma Vertriest
5:10-5:20 PM	O-074	Novel (Re)Emerging Substance? 5F-ADB Detection in Postmortem Toxicology	Kevin Shanks
5:20-5:30 PM	O-075	From Ionic Homeostasis Modulation to Oxidative Stress: Toxicity Pathways of 4th-Generation Synthetic Cathinones	Giorgia Sprega

PLATFORM SESSION 8: ALTERNATIVE MATRICES II: HAIR & NAIL TUESDAY, SEPTEMBER 22 | INTERNATIONAL SOUTH BALLROOM MODERATORS: ROBERT KRONSTRAND & MANDI MOHR			
TIME	**#**	**TITLE**	**PRESENTER**
4:00-4:10 PM	O-076	Development and Validation of a Sensitive LCMS^3 Method for the Quantitative Analysis of Ethyl Glucuronide in Hair	Ryan Paulsen
4:10-4:20 PM	O-077	LC-MS/MS Analysis of Drugs of Abuse in Human Nail Specimens: A Forensic Study of Drug Users in Jordan	Abdel-Qader Asad
4:20-4:30 PM	O-078	Pharmaco-Toxicological Discussion on the Incorporation in Hair of Four Doping Agents Consumed at an Identical Dose	Laurie Gheddar
4:30-4:40 PM	O-079	Can the Macujo Method Evade Drug Detection in Hair? An Experimental Evaluation on Positive Samples	Marine Deville
4:40-4:50 PM	O-080	The Power of Chiral Amphetamine Analysis in Hair: Telling Therapy Compliance From Illicit Amphetamine Use	Liesl K Janssens

PLATFORM SESSION 8: ALTERNATIVE MATRICES II: HAIR & NAIL TUESDAY, SEPTEMBER 22 | INTERNATIONAL SOUTH BALLROOM MODERATORS: ROBERT KRONSTRAND & MANDI MOHR			
TIME	**#**	**TITLE**	**PRESENTER**
4:50-5:00 PM	O-081	Uncovering Drug Use at the Time of a Criminal Offense: Segmental Hair Analysis in a Forensic Psychiatric Population	Ana Simão
5:00-5:10 PM	O-082	Orbitrap HRMS-Based Workflow for Hair Analysis to Reveal Hidden Patterns of NPS Exposure	Giorgia Ferrero
5:10-5:20 PM	O-083	Optimization and Validation of a Method for Detecting Single Dose Drug Exposure in Hair: Application to Authentic Specimens	Kaylyn Keith
5:20-5:30 PM	O-084	Development and Validation of a Homogeneous Enzyme Immunoassay Screen for PCP Analysis in Hair	Neil Stowe

PLATFORM SESSION 9: POSTMORTEM I WEDNESDAY, SEPTEMBER 23 | INTERNATIONAL NORTH BALLROOM MODERATORS: REBECCA HARTMAN & PETER MASKELL			
TIME	**#**	**TITLE**	**PRESENTER**
8:00-8:10 AM	O-085	Brain:Femoral Blood Ratios of Amphetamine, Benzoylecgonine, Cocaethylene, Cocaine, Methamphetamine, and Fentanyl	Grayce Behnke
8:10-8:20 AM	O-086	Simethicone-Associated Pharmacobezoar Formation in Fatal Multi-Drug Overdose: Clinical and Toxicological Insights	Karolina Nowak
8:20-8:30 AM	O-087	Is Red Bone Marrow a Reliable Matrix for Estimating Blood Ethanol in Decomposed Bodies? A Pilot Study with Focus on the Lipid Fraction	Sara Casati
8:30-8:40 AM	O-088	Contrasting Indicators: Aligning Overdose Mortality and Drug Seizure Trends in Arkansas	Mary Lynn Heffington
8:40-8:50 AM	O-089	Ongoing Decline in Fentanyl-Related Mortality in the United States: Evidence for Contribution From a Supply-Driven Inflection Within a Complex Public Health Response	Barry Logan
8:50-9:00 AM	O-090	Evidence of Multifactorial Ketoacidosis in a Postmortem Toxicology Case: Resolving Discrepant Inter-Matrix Ethanol Findings Through Beta-Hydroxybutyrate Quantification and Case History	Ayako Chan-Hosokawa

PLATFORM SESSION 9: POSTMORTEM I WEDNESDAY, SEPTEMBER 23 | INTERNATIONAL NORTH BALLROOM MODERATORS: REBECCA HARTMAN & PETER MASKELL			
TIME	**#**	**TITLE**	**PRESENTER**
9:00-9:10 AM	O-091	Warning from Madrid on the Dangers of Chemsex: A Toxicological Cohort Study of 35 Autopsy Cases (2017-2025)	María-Antonia Martínez-González
9:10-9:20 AM	O-092	From Evidence to Artifact? Matrix-Dependent Drug Detection and Formalin-Associated Redistribution in Fixed Postmortem Tissues	Telmo Gomes
9:20-9:30 AM	O-093	Postmortem Blood Lead Profiles in Non-Firearm Related Deaths and Firearm Related Deaths	Prentiss Jones
9:30-9:40 AM	O-094	Fatal EDTA Toxicity Following Chelation Therapy	Yi Ju Yao
9:40-9:50 AM	O-095	Prevalence of Naloxone with Opioids and Non-Opioids in Dallas County	Lindsay Glicksberg
9:50-10:00 AM	O-096	Toxicological Evaluation of Fentanyl-Involved Pediatric Homicides	Elba Arango

PLATFORM SESSION 10: NEW TECHNOLOGIES, AI & DATA SCIENCE I WEDNESDAY, SEPTEMBER 23 | INTERNATIONAL SOUTH BALLROOM MODERATORS: ANDREA STEUER & JERI ROPERO-MILLER			
TIME	**#**	**TITLE**	**PRESENTER**
8:00-8:10 AM	O-097	Data Mining with OpenMS for the Retrospective Identification of Out-of-Scope Analytes	Madison Schackmuth
8:10-8:20 AM	O-098	Development and Implementation of an In-House Built and Customized Laboratory Information Management System	Tyler Devincenzi
8:20-8:30 AM	O-099	Multimodal Bayesian Retrieval of Obscured Drug-Related Content for Forensic Informatics and Clinical Toxicology	Hande McGinty
8:30-8:40 AM	O-100	Implementation of Artificial Intelligence Tools in a Forensic Toxicology Laboratory: Enhancing Efficiency and Quality Management	Wojciech Lechowicz
8:40-8:50 AM	O-101	Chromatographic Peak Detection and Integration by Supervised Machine Learning using Convolutional Neural Networks	Mark Parkin
8:50-9:00 AM	O-102	PhaT-GPT (Pharmacological Toxicity Assessment by Global Pill Testing): Application of Activity-Based Opioid Screening in Drug Checking	Marthe Vandeputte

PLATFORM SESSION 10: NEW TECHNOLOGIES, AI & DATA SCIENCE I WEDNESDAY, SEPTEMBER 23 | INTERNATIONAL SOUTH BALLROOM MODERATORS: ANDREA STEUER & JERI ROPERO-MILLER			
TIME	**#**	**TITLE**	**PRESENTER**
9:00-9:10 AM	O-103	Navigating the New Frontier: Method Development Challenges in Fast Oral Fluid Analysis by DART-MS/MS	Apolline Derendinger
9:10-9:20 AM	O-104	Automated R-Based Workflow for GC-MS Impurity Profiling: Application to Methamphetamine Datasets	Alasoul Saif
9:20-9:30 AM	O-105	Assessing DRUID's Ability to Distinguish Between Alcohol and Cannabis-Induced Impairment Using Machine Learning	Sumandeep Rana
9:30-9:40 AM	O-106	Endogenous Sleepiness Markers Could Aid Forensic Routine DUI Case Interpretation	Michael Scholz
9:40-9:50 AM	O-107	Assistive vs Autonomous Applications of Artificial Intelligence and Machine Learning (AI/ML) in Forensic Toxicology: A U.S. Admissibility-Focused Framework Preserving Expert Judgment	MJ Menendez
9:50-10:00 AM	O-108	Python-Accelerated Processing of TraceFinder™ Method Validation Data per ANSI/ASB Standard 036	Szabolcs Sofalvi
10:00-10:30 AM - MORNING BREAK			

PLATFORM SESSION 11: POSTMORTEM II WEDNESDAY, SEPTEMBER 23 | INTERNATIONAL NORTH BALLROOM MODERATORS: JENNIFER SCHUMANN & RUTH WINECKER			
TIME	**#**	**TITLE**	**PRESENTER**
10:30-10:40 AM	O-109	Emerging Threats in Synthetic Opioids: A Case of Fatal Intoxication Involving Spirochlorphine (R-6890)	Sophia Wrbas
10:40-10:50 AM	O-110	What's in My Meth? Investigating Incidental Findings in Methamphetamine-Positive Postmortem Toxicology Drug Screens	Crystal Arndt
10:50-11:00 AM	O-111	Emergence of Medetomidine in San Francisco Forensic Toxicology Casework	Maria Sarkisian
11:00-11:10 AM	O-112	Kratom-Related Fatalities With Opioid Co-Detection: Toxicological Complexity and Medico-Legal Challenges	Giulia Bambagiotti
11:10-11:20 AM	O-113	Variability in Cause of Death Attribution in Nitazene-Related Fatalities	Jessica Kent

PLATFORM SESSION 11: POSTMORTEM II WEDNESDAY, SEPTEMBER 23 | INTERNATIONAL NORTH BALLROOM MODERATORS: JENNIFER SCHUMANN & RUTH WINECKER			
TIME	**#**	**TITLE**	**PRESENTER**
11:20-11:30 AM	O-114	Synthetic Cannabinoid Symptomology in North Carolina Deaths	Laura Friederich
11:30-11:40 AM	O-115	Hidden in Plain Sight: Lessons From NPS Fatalities in Luxembourg	Stefania Oliverio
11:40-11:50 AM	O-116	Where Did the Blue Intestine Come From? A Case Report of a Fatal Combined Intoxication With Cocaine, γ-Hydroxybutyric Acid (GHB), Morphine, and Rilmazolam	Wiebke Rudolph-Allritz
11:50 AM-12:00 PM	O-117	Potential Fatal Interaction Between Psilocin, Phosphodiesterase Inhibition, and Adenosine Antagonism: A Case Report and Mechanistic Hypothesis	Robert Moore

PLATFORM SESSION 12: NEW TECHNOLOGIES, AI & DATA SCIENCE II WEDNESDAY, SEPTEMBER 23 | INTERNATIONAL SOUTH BALLROOM MODERATORS: SUMANDEEP RANA & MARIA SARKISIAN			
TIME	**#**	**TITLE**	**PRESENTER**
10:30-10:40 AM	O-118	A Data Processing Framework for the Untargeted Analysis of High-Resolution Mass Spectrometry Data to Enable NPS Detection and Metabolomics in Forensic Casework	Samantha Swan
10:40-10:50 AM	O-119	Interpretive Toxicology: Fitting Probability Distributions to Post-Mortem Drug Case Data	Peter D Maskell
10:50-11:00 AM	O-120	Don't Let AI Think for You; Make AI Work for You	Alexander Giachetti
11:00-11:10 AM	O-121	Leveraging Open-Source Computational Tools to Generate Contrived Data for Validating Data Processing Systems	William Slade
11:10-11:20 AM	O-122	High-Resolution Mass Spectrometry-Based Molecular Networking Workflow for Untargeted Detection of Emerging Nitazenes in Seized Materials	Alexandre Godoi

PLATFORM SESSION 12: NEW TECHNOLOGIES, AI & DATA SCIENCE II WEDNESDAY, SEPTEMBER 23 | INTERNATIONAL SOUTH BALLROOM MODERATORS: SUMANDEEP RANA & MARIA SARKISIAN			
TIME	**#**	**TITLE**	**PRESENTER**
11:20-11:30 AM	O-123	Behavioral Patterns and Social Media Explorations for Toxicology using Neurosymbolic AI Approaches: A Case Study for Drug Misuse	Patrick Hancock
11:30-11:40 AM	O-124	Development and Validation of a Deep-Learning Screening Method for NPS in Routine Forensic Casework	Samantha Swan
11:40-11:50 AM	O-125	Risk-Based Evaluation of AI Responses to NPS Synthesis-Related Queries	Karen Araujo
11:50 AM-12:00 PM	O-126	Development and Evaluation of a Custom Made HRMS Library for Screening Novel Psychoactive Substances in Blood	Jari Rubbens
12:00-1:30 PM - LUNCH			

PLATFORM SESSION 13: DRUG FACILITATED CRIME & OTHER TOPICS WEDNESDAY, SEPTEMBER 23 | INTERNATIONAL NORTH BALLROOM MODERATORS: DIMITRI GEROSTAMOULOS & CONNIE ALEXIA LEWIS			
TIME	**#**	**TITLE**	**PRESENTER**
1:30-1:40 PM	O-127	Lab-Quality Portable Microfluidic Platform for Objective Semi-Quantitative On-Site Drug Screening in Urine	Sora An
1:40-1:50 PM	O-128	Identification of Δ9-THCB, Δ9-THCH, and Δ9-THCP Phase I Biomarkers and Minor Metabolites with Human Liver Microsomes	Kathryn James
1:50-2:00 PM	O-129	Evaluation of Acetone as a Biomarker for Alcohol Involvement in Drug-Facilitated Sexual Assault Cases	Mellissa Gentry
2:00-2:10 PM	O-130	Analytical Confirmations of Emergency-Department Intoxications from April 2022 – March 2026 in Victoria, Australia	Jared Castle
2:10-2:20 PM	O-131	Can a Single Dose Leave a Trace? Interpretation of Sedating Antihistamines in Hair After Single-Dose Exposure and Application to Drug-Facilitated Crime Cases	Nadia Arbouche
2:20-2:30 PM	O-132	Oral Fluid Test Results After Acute and Chronic Dosing of a Topical Hemp Product in Uncontrolled and Controlled Settings	Halle Thomas

PLATFORM SESSION 13: DRUG FACILITATED CRIME & OTHER TOPICS WEDNESDAY, SEPTEMBER 23 | INTERNATIONAL NORTH BALLROOM MODERATORS: DIMITRI GEROSTAMOULOS & CONNIE ALEXIA LEWIS			
TIME	**#**	**TITLE**	**PRESENTER**
2:30-2:40 PM	O-133	Establishment of a National Drug Checking Laboratory: Support Toxicological Surveillance in Scotland	Lorna Nisbet
2:40-2:50 PM	O-134	Scopolamine Detection Following N-Butylscopolamine Administration: Toxicological Considerations in DFC and DFSA Cases	Natalia Pérez-Hernández
2:50-3:00 PM	O-135	Integrating Self-Reported and Biological Measures to Assess Psychoactive Substance Use Among Medical Students in Italy: The NO PANIC Study	Valentina Martini
3:00-3:10 PM	O-136	Neurochemical Effects of Chronic Ketamine Exposure: Insights from Mass Spectrometry Imaging and Metabolomics	Weihao Fan
3:10-3:20 PM	O-137	Evaluation of Sexual Assault Cases in Houston: A Six-Year Update (2019-2024)	Jessica Ayala
3:20-3:30 PM	O-138	Drug Residue Analysis of Discarded Paraphernalia: 12-Month Spatio-Temporal Patterns From South Australia	Brock Peake

PLATFORM SESSION 14: NPS III - SYNTHETIC OPIOIDS WEDNESDAY, SEPTEMBER 23 | INTERNATIONAL SOUTH BALLROOM MODERATORS: DANI MATA & ROB MOORE			
TIME	**#**	**TITLE**	**PRESENTER**
1:30-1:40 PM	O-139	Rapid LC-HRMS-Based Nontargeted Analysis of Emerging Nitazene and Orphine Opioids in Human Urine	Akshita Verma
1:40-1:50 PM	O-140	Investigation of Methylenedioxynitazene Metabolism in Human Hepatocytes: Identification of Metabolic Biomarkers Using LC-HRMS/MS	Alida Likey
1:50-2:00 PM	O-141	Fatal Intoxication with the Nitazene N-Pyrrolidino Fluetonitazene: Quantification, Metabolism and μ-Opioid Receptor Affinity	Katharina Elisabeth Grafinger
2:00-2:10 PM	O-142	Small Change, Big Difference? In Vitro Characterization of a Series of 5-Cyano Desnitazene Opioids or "Cyanazenes"	Inez Lambrecht
2:10-2:20 PM	O-143	Inhibition of Cardiac Voltage-Gated Ion Channels by New Synthetic Opioids	Caitlyn Norman
2:20-2:30 PM	O-144	Comprehensive Metabolic Characterization and Enantioselective Profiling of N-Propionitrile Chlorphine, an Emerging Orphine-Type Synthetic Opioid	Omayema Taoussi

PLATFORM SESSION 14: NPS III - SYNTHETIC OPIOIDS WEDNESDAY, SEPTEMBER 23 | INTERNATIONAL SOUTH BALLROOM MODERATORS: DANI MATA & ROB MOORE			
TIME	**#**	**TITLE**	**PRESENTER**
2:30-2:40 PM	O-145	Phase I Metabolism Study on the New Synthetic Opioid Methiodone Involved in Seven Intoxication Cases	Johannes Kutzler
2:40-2:50 PM	O-146	Navigating the Evolving Novel Synthetic Opioid Landscape	Kimberly Yacoub Mansour
2:50-3:00 PM	O-147	Can Molecular Docking and Dynamics Predict Opioids Potency? Pharmacological and Computational Insights From Methylenedioxynitazene	Diletta Berardinelli
3:00-3:10 PM	O-148	Metabolism Study and Quantification of the New Synthetic Opioid Methiodone in Clinical and Forensic Cases via LC-MS/MS	Johannes Rassbach
3:10-3:20 PM	O-149	Comprehensive LC-MS/MS Analysis of Synthetic Cocaine Derivatives: From Stereoisomer Differentiation to Real-World Forensic Applications	Tom Richard Sundermann
3:20-3:30 PM	O-150	Detection of N-propionitrile Chlorphine (Cychlorphine) Throughout the United States in Healthcare Patient Urine Samples	David M Schwope
3:30-4:00 PM - AFTERNOON BREAK			

PLATFORM SESSION 15: CLINICAL & ENVIRONMENTAL WEDNESDAY, SEPTEMBER 23 | INTERNATIONAL NORTH BALLROOM MODERATORS: SAM JOUBERT & LEA WAGMANN			
TIME	**#**	**TITLE**	**PRESENTER**
4:00-4:10 PM	O-151	Integrated Clinical-Toxicological Surveillance of Psychoactive Substances in Open Drug Scenes in Sao Paulo, Brazil	Náthaly Cristine Bueno Faria dos Santos
4:10-4:20 PM	O-152	Prevalence Rates, Concordance, and Cognitive Correlates of Biochemically-Verified Cannabis Use in a Large Cohort of Healthy Adolescents Over Time	Natasha Wade
4:20-4:30 PM	O-153	Longitudinal Assessment of Alcohol Biomarkers for Differentiating Self-Reported Drinking Behaviors	Munchelou Gomonit
4:30-4:40 PM	O-154	Targeted Toxicology: Establishing Overdose Biosurveillance in Vermont with a 75-Compound Panel	Suzanne Stanton
4:40-4:50 PM	O-155	Comprehensive Drug Testing in Dried Blood Spots for Multi-Year Surveillance of Unregulated Drug Use in Rhode Island	Adina Badea

PLATFORM SESSION 15: CLINICAL & ENVIRONMENTAL WEDNESDAY, SEPTEMBER 23 | INTERNATIONAL NORTH BALLROOM MODERATORS: SAM JOUBERT & LEA WAGMANN			
TIME	**#**	**TITLE**	**PRESENTER**
4:50-5:00 PM	O-156	LC-MS/MS QTOF Analysis of River Water Identifies Contaminants of Environmental Concern by Non-Targeted Profiling	Sarah Monti
5:00-5:10 PM	O-157	Wastewater-Based Epidemiology of Prescription Opioids in Reykjavík, Iceland Between 2024–2025	Adam Bauer
5:10-5:20 PM	O-158	Toxicological Profiling of Liberian Local Remedies: Implications for Health and Safety	Noemi Mangiamele
5:20-5:30 PM	O-159	Driving Impairment and TDM Adjustment Pre and Post Liver Transplant	Douglas E Rohde

PLATFORM SESSION 16: BEST PRACTICES WEDNESDAY, SEPTEMBER 23 | INTERNATIONAL SOUTH BALLROOM MODERATORS: SARAH WILLE & MARC LEBEAU			
TIME	**#**	**TITLE**	**PRESENTER**
4:00-4:10 PM	O-160	2026 Update on Standards Development Activities in Forensic Toxicology	Jennifer F Limoges
4:10-4:20 PM	O-161	A Novel Model to Discern Exogenous Gamma-Hydroxybutyric Acid (GHB) in Postmortem Cases	Sue Pearring
4:20-4:30 PM	O-162	GHB in Québec (Canada): The Data and the Story	Edith Viel
4:30-4:40 PM	O-163	Streamlined Approaches to Measurement Uncertainty Evaluation in a 200+ Drug Analysis	Justin Dang
4:40-4:50 PM	O-164	Mathematics-Based Best Practices and Software Tools for the Method of Standard Addition	Jocelyn V Abonamah

PLATFORM SESSION 16: BEST PRACTICES WEDNESDAY, SEPTEMBER 23 | INTERNATIONAL SOUTH BALLROOM MODERATORS: SARAH WILLE & MARC LEBEAU			
TIME	**#**	**TITLE**	**PRESENTER**
4:50-5:00 PM	O-165	Application of Risk Management to Analytical Method Validations	Michael Baldwin
5:00-5:10 PM	O-166	Multi-Factor Fermentation Experiment in Ante-Mortem Blood Alcohol Testing	Sarah Douglas
5:10-5:20 PM	O-167	Δ8-THC in the Marketplace and the Laboratory: Δ8-THC Product Labeling Accuracy and Standard Urinary Drug Testing	Lakshmi Kumar
5:20-5:30 PM	O-168	Lessons Learned the Hard Way: A Multi-Year Implementation Case Study in a Forensic Toxicology Laboratory	Lisa Branch

PLATFORM SESSION 17: NPS IV THURSDAY, SEPTEMBER 24 | INTERNATIONAL NORTH BALLROOM MODERATORS: MARILYN HUESTIS & MARTHE VANDEPUTTE			
TIME	**#**	**TITLE**	**PRESENTER**
8:00-8:10 AM	O-169	Differentiation, Quantitation, and Stability Assessment of Newly Emergent Kratom Alkaloids in Human Blood via High-Resolution Mass Spectrometry	Lauren Eccarius
8:10-8:20 AM	O-170	Phenazolam Emergence: A Case Series of Fatal and Nonfatal Exposures	Brianna Stang
8:20-8:30 AM	O-171	Toxicokinetics of Four N,N-Dimethyltryptamine Derivatives Studied in Human in Vitro Systems and in Vivo by Means of Zebrafish Embryos	Gloria Daziani
8:30-8:40 AM	O-172	Dihydro-7-Hydroxy Mitragynine (MGM-15) – A Novel Synthetic Kratom Alkaloid Implicated in Fatalities	Alex Krotulski
8:40-8:50 AM	O-173	The Pharmacokinetics of Clonazafone and Its Analytical Challenges – an In-Vivo Experiment	Jonas Malzacher
8:50-9:00 AM	O-174	Development of a Targeted Dynamic Analytical Strategy for the Detection of New Psychoactive Substances in Forensic Toxicology	Gabriela de Paula Meirelles

PLATFORM SESSION 17: NPS IV THURSDAY, SEPTEMBER 24 | INTERNATIONAL NORTH BALLROOM MODERATORS: MARILYN HUESTIS & MARTHE VANDEPUTTE			
TIME	**#**	**TITLE**	**PRESENTER**
9:00-9:10 AM	O-175	Detection of Etomidate and Its Metabolites in Human Hair	Hooi Yan Moy
9:10-9:20 AM	O-176	A Comprehensive LC-MS/MS Triage Workflow for 135 Forensic Targets in Qatar: Optimizing Solid-Phase Extraction for the Preservation of Highly Labile Ester-Linked Synthetic Cannabinoids	Tarek Mahdy
9:20-9:30 AM	O-177	BTMPS in Biological Samples in the Absence of Illicit Drugs	Laureen Marinetti
9:30-9:40 AM	O-178	Integrating Predictive Fragmentation Into HRMS Workflows for the Identification of Metabolite Biomarkers Phase I and Phase II and the Extension of Detection Windows in Forensic Toxicology	Jose Manuel Matey
9:40-9:50 AM	O-179	When Structure Matters: Immunoassay Cross-Reactivity of Synthetic Cocaine Derivatives	Max Dautert
9:50-10:00 AM	O-180	Potent and Irreversible? Evaluating the Monoamine Oxidase Inhibition Profile of Eight Psychedelics of the Cyclopropylamine Class	Lea Wagmann

PLATFORM SESSION 18: ANALYTICAL III THURSDAY, SEPTEMBER 24 | INTERNATIONAL SOUTH BALLROOM MODERATORS: DAYONG LEE & LANA BROCKBALS			
TIME	**#**	**TITLE**	**PRESENTER**
8:00-8:10 AM	O-181	Is "Levamisole" Always Levamisole in Adulterated Cocaine? Answer Through Enantioselective High-Performance Liquid Chromatography and Supercritical Fluid Chromatography Coupled With Tandem Mass Spectrometry	Elizabeth Lomokei Pobee
8:10-8:20 AM	O-182	A Hallucinogenic Tryptamine Panel for the Modern Era and its Application to Forensic Toxicology Casework	Sarah Doumit
8:20-8:30 AM	O-183	Qualitative Detection of MDMB-4en-PINACA and 5F-ADB Metabolites Using HPLC-DAD and GC-QQQ/MS	Ahmed Alnazer
8:30-8:40 AM	O-184	Green Analytical Toxicology – Development of a Sustainable Qual-Quant Workflow Exemplified for Neuroleptics and Antidepressants in Human Plasma	Rabekka Thuraiveerasingam
8:40-8:50 AM	O-185	Switching It Up: A Novel Method Using Switchable Hydrophilicity Solvent Liquid-Liquid Microextraction and LC-MS/MS for the Analysis of Traditional and Novel Stimulants in Blood	Eduardo de Campos
8:50-9:00 AM	O-186	Identification of Cannabinoids Related to Delta-9-THC Through Derivatization and GC-MS Analysis	James DeFrancesco

PLATFORM SESSION 18: ANALYTICAL III THURSDAY, SEPTEMBER 24 | INTERNATIONAL SOUTH BALLROOM MODERATORS: DAYONG LEE & LANA BROCKBALS			
TIME	**#**	**TITLE**	**PRESENTER**
9:00-9:10 AM	O-187	Phenol in Postmortem Heart Blood: Produced by Decomposition or Exogenous Intake During Life?	Chang Jing
9:10-9:20 AM	O-188	Evaluating Strategies for Low-Level Quantification of THC Isomers and Metabolites in Whole Blood by Conventional GC-MS Instrumentation	Amanda Rigdon
9:20-9:30 AM	O-189	Identification and Chiral Separation of N-Ethylpentedrone (NEP) and its Metabolites by Capillary Electrophoresis-Mass Spectrometry Using Cyclodextrins as Chiral Selectors	Francesco Tavoletta
9:30-9:40 AM	O-190	Chiral Separation and Identification of Ketamine, Norketamine, and Medetomidine: Method Development and Application to Forensic Samples	Savannah Baker
9:40-9:50 AM	O-191	Z/E-Methoxime Isomers in Gas Chromatography-Quadrupole Mass Spectrometry Analysis of C6-keto-Opioids in Human Urine as Their Methoxime- and Acyl-Derivatives	David Barajas
9:50-10:00 AM	O-192	Performance Comparison of LDTD-Coupled Orbitrap and Triple Quadrupole Mass Spectrometry for Urinary Screening of 23 Drugs Using Unified Extraction Method	Serge Auger
10:00-10:30 AM - MORNING BREAK			

PLATFORM SESSION 19: LIGHTNING I - NEW TECHNOLOGIES, NPS & ANALYTICAL THURSDAY, SEPTEMBER 24 | INTERNATIONAL NORTH BALLROOM MODERATORS: CHRISTOPHE STOVE & SUE PEARRING			
TIME	**#**	**TITLE**	**PRESENTER**
10:30-10:35 AM	L-001	From Fragments to Framework: Pre- and Post-Implementation Analysis of CLIO, an In-House Laboratory Information Management System	Belle Lunasco
10:35-10:40 AM	L-002	Evaluation of in Silico ADME and Toxicity Predictions for Novel Psychoactive Substances Using Percepta	Ashef Ameer
10:40-10:45 AM	L-003	Time-Dependent Distribution in Tissue, Blood and Urinary of Three Emerging Ketamine Analogues: 2-MDCK, 2-FXE, and 2-oxo-PCE in Rodent Models	Weihao Fan
10:45-10:50 AM	L-004	Increasing Carfentanil Positivity in Clinical Urine Specimens from 2024 to 2025	Alicia Bland
10:50-10:55 AM	L-005	A Methadone and Benzodiazepine Nal-Von-Minden Screening Test Could Not Be Confirmed: A Belgian NPS Puzzle of 4 Lethal Cases Involving Methiodone	Katleen Van Uytfanghe
10:55-11:00 AM	L-006	When Standards Don't Exist, the Samples Do: Navigating Diastereomeric Complexity in Mitragynoid LC-MS/MS Analysis	Danai T Taruvinga

PLATFORM SESSION 19: LIGHTNING I - NEW TECHNOLOGIES, NPS & ANALYTICAL THURSDAY, SEPTEMBER 24 | INTERNATIONAL NORTH BALLROOM MODERATORS: CHRISTOPHE STOVE & SUE PEARRING			
TIME	**#**	**TITLE**	**PRESENTER**
11:00-11:05 AM	L-007	Prevalence of Kratom in Postmortem Cases, Impaired Drivers, and Drug-Facilitated Sexual Assault Cases within San Francisco	Stephanie Basiliere
11:05-11:10 AM	L-008	Early Identification of N-Propionitrile Chlorphine, aka cychlorphine, in a Western U.S. Jurisdiction	Luke N Rodda
11:10-11:15 AM	L-009	Identification and Quantification of Four Etomidate Analogues in Hair Samples by LC-MS/MS	Yinyin Dai
11:15-11:20 AM	L-010	Designer Benzodiazepines – Is There a Need to Validate a Quantitative Method	Connie Lewis
11:20-11:25 AM	L-011	Identification of ADB-5en-HEXINACA and Other Synthetic Cannabinoids by GC-MS in Adulterated Low-THC Cannabis Products	Enrico Gerace
11:25-11:30 AM	L-012	Enzymatic Hydrolysis of Psilocin-O-glucuronide for Screening of Psilocin in Human Urine by Liquid Chromatography-Triple Quadrupole Tandem Mass Spectrometry	David Barajas

PLATFORM SESSION 19: LIGHTNING I - NEW TECHNOLOGIES, NPS & ANALYTICAL THURSDAY, SEPTEMBER 24 | INTERNATIONAL NORTH BALLROOM MODERATORS: CHRISTOPHE STOVE & SUE PEARRING			
TIME	**#**	**TITLE**	**PRESENTER**
11:30-11:35 AM	L-013	When the Sample Isn't Real: A Tiered Screening and LC-QTOF-MS/MS Confirmation Strategy for Detection of Diluted, Adulterated, Substituted, and Synthetic Urine Samples	Ashraf Mina
11:35-11:40 AM	L-014	Sensitive Detection of the Alcohol Biomarker Ethylated Phosphorylcholine (EtOChoP) in Urine by a Reversed-Gradient Reversed-Phase LC-MS/MS and Comparison to Elimination of EtG in Urine in a Pilot Single-Subject Drinking Study	Philipp Eberhardt
11:40-11:45 AM	L-015	Drug Screen Suite: A Streamlined LC-HRMS Workflow for Routine and Post-Mortem Toxicological Analysis	Artem Filipenko
11:45-11:50 AM	L-016	Recommendations for Expanding the Scope of an Existing LC-MS/MS Method	Samantha Herbick

PLATFORM SESSION 20: LIGHTNING II - DRUGS & DRIVING, POSTMORTEM, CLINICAL & ENVIRONMENTAL, & OTHER TOPICS THURSDAY, SEPTEMBER 24 | INTERNATIONAL SOUTH BALLROOM MODERATORS: SVANTE VIKINGSSON & JASMINE MAXWELL			
TIME	**#**	**TITLE**	**PRESENTER**
10:30-10:35 AM	L-017	Evaluation of Blood Fentanyl Concentrations in Wisconsin Drug-Impaired Driving Cases from 2016 to 2025	Kayla Neuman
10:35-10:40 AM	L-018	Assessment of Response of the Intoxilyzer® 9000 to Volatiles of Forensic Relevance	Bradley Dafoe
10:40-10:45 AM	L-019	Evolving Trends in Impaired Driving: A Data-Driven Analysis	Amanda Mohr
10:45-10:50 AM	L-020	Enantioselective Determination of Amphetamine and Methamphetamine in Paired Postmortem Matrices by GC–MS/MS	Robin Hahn
10:50-10:55 AM	L-021	Fatal Phencyclidine Intoxication	Teresa Gray
10:55-11:00 AM	L-022	The Detection of N-Propionitrile Orphine in a Post-Mortem Urine Sample in the United Kingdom	Rebecca Wood

PLATFORM SESSION 20: LIGHTNING II - DRUGS & DRIVING, POSTMORTEM, CLINICAL & ENVIRONMENTAL, & OTHER TOPICS THURSDAY, SEPTEMBER 24 | INTERNATIONAL SOUTH BALLROOM MODERATORS: SVANTE VIKINGSSON & JASMINE MAXWELL			
TIME	**#**	**TITLE**	**PRESENTER**
11:00-11:05 AM	L-023	Sudden Death in the Context of Clenbuterol and Anabolic Steroid Use	M José Burgueño
11:05-11:10 AM	L-024	Elevated Postmortem THC Concentrations Following Traumatic Deaths	Matthew Juhascik
11:10-11:15 AM	L-025	Tissue Distribution of Daridorexant in a Forensic Autopsy Case	Brian Waters
11:15-11:20 AM	L-026	The Final Dose: Postmortem Toxicology in Suicide Cases	Sara Howard
11:20-11:25 AM	L-027	Trends in Drug Positivity in Correctional Facility Deaths in Alabama	Rebecca Smith
11:25-11:30 AM	L-028	Thallium Intoxication vs. Differential Diagnosis of Guillain-Barré-Syndrome – Report of a Series of Poisonings	Martin Juebner

PLATFORM SESSION 20: LIGHTNING II - DRUGS & DRIVING, POSTMORTEM, CLINICAL & ENVIRONMENTAL, & OTHER TOPICS THURSDAY, SEPTEMBER 24 | INTERNATIONAL SOUTH BALLROOM MODERATORS: SVANTE VIKINGSSON & JASMINE MAXWELL			
TIME	**#**	**TITLE**	**PRESENTER**
11:30-11:35 AM	L-029	Forensic Evidence of a Significant Increase in Recreational Ketamine Use on the Spanish Mediterranean Coast	Alberto Cordoba Sola
11:35-11:40 AM	L-030	Application of Fortified Hair as Quality Control Sample in Multi-drug Analysis	Si Jia Chan
11:40-11:45 AM	L-031	Cross-Reactivity of Risperidone in Immunochemical Urine Screening for Fentanyl: A Case Study within the Italian Fentanyl Prevention Plan	Annagiulia Di Trana
11:45-11:50 AM	L-032	Customized Toxicological Criteria for the Korean Population: Blood Concentrations and Clinical Symptoms in Acute Poisoning	Ilchung Shin
12:00-2:00 PM - LUNCH			

POSTER SESSION 1 TUESDAY, SEPTEMBER 22 | 12:00-1:30PM | SALON LOBBY MODERATORS: ALICE SEYWRIGHT & MARISOL CASTANETO		
#	**TITLE**	**PRESENTER**
P-100	Development and Validation of Automated Homogeneous Immunoassays for Opiates and Oxycodone in Oral Fluid: Integrating Structural Biology Practices into Antibody Engineering and Characterization	Erik Cook
P-101	Development of an Automated Pretreatment Method for Cannabinoids and Related Compounds in Serum Using ATLAS-LEXT	Musashi Abe
P-102	Evaluation of a Second-Generation Nitazene Lateral Flow Test Strip Using an Expanded Analogue Panel	Victoria Marland
P-103	Conduct and Detect: A One-Step Conductometric Screen for Fraudulent Urine Specimens	Melike Aydoğdu
P-104	Validation of an Automated Extraction for the Quantitation and Identification of Cannabinoids in Oral Fluid for DUI Casework	Tara Federico
P-105	Into the Unknown: Evaluating Time of Flight Drug Screening Against Gas Chromatography Mass Spectrometry Drug Screening	Kahleah Pell
P-106	Evaluation of Colorimetric, Microcrystalline, and LC-MS/MS Methods for Emerging Synthetic Drug Adulterants: Addressing Specificity Gaps in Modern Drug Detection	Katherine Davis
P-107	Comparison of Enzymatic Hydrolysis and Formic Acid Acidification for Improved Detection of Drug Metabolites in Non-Fatal Overdose Urine by LC-QToF-MS	Abby Veeser

POSTER SESSION 1 TUESDAY, SEPTEMBER 22 | 12:00-1:30PM | SALON LOBBY MODERATORS: ALICE SEYWRIGHT & MARISOL CASTANETO		
#	**TITLE**	**PRESENTER**
P-108	Development of Conditions for HPLC Chromatography of the Substance Dimetindene Maleate and Its Toxic Chemical Degradation Products	Welchinska Olena
P-109	A Rapid and Highly Sensitive Analytical Method for Comprehensive Determination of Cocaine and Hydroxy Metabolites in Hair	Alexis Palma
P-110	A QuEChERS LC/MS/MS Comprehensive Method for Abuse Drugs, Medications, and Pesticide Detection on Biological Samples in the Toxicology Unit of the Costa Rican Forensic Science Laboratory Department	Pablo Castillo
P-111	Molecular Mirrors: Enantioselective LC–MS/MS Differentiation of Amphetamine and Methamphetamine in Whole Blood	Telmo Gomes
P-112	LC-MS/MS Methods for Alcohol Biomarkers in Urine and Whole Blood	Samantha Herbick
P-113	Comparative Evaluation of High-Resolution Mass Spectrometry Acquisition Strategies for the Detection and Analysis of Illicit Drug Substances	Nayan Mistry
P-114	A Ready-to-Run Workflow for Quantitating 80 Drugs of Abuse in Whole Blood with the TSQ Certis Triple Quadrupole MS	Jingshu Guo
P-115	Validation of the Neogen® Gabapentin ELISA Kit for Whole Blood and Urine Specimens	Chloe Rosenberger

POSTER SESSION 1 TUESDAY, SEPTEMBER 22 | 12:00-1:30PM | SALON LOBBY MODERATORS: ALICE SEYWRIGHT & MARISOL CASTANETO		
#	**TITLE**	**PRESENTER**
P-116	LC–MS/MS Analysis of Caffeine in Electronic Cigarettes and Its Pharmacokinetic Application to Biological Samples	Kanta Noguchi
P-117	Semi-Synthetic Cannabinoids: The Newest Challenge in Analyzing THC Isomers	Jared Burkhart
P-118	Simultaneous Analysis of 27 Multi-Class Antidiabetic Drugs in Blood Using LC-MS/MS	Toshinari Ishii
P-119	Fast Screening for 44 Illicit Drugs in Urine by UHPLC/MS/MS	Autumn Payne
P-120	Validated Liquid Chromatography-Tandem Mass Spectrometry Method for Urinary Gonadotrophic-Releasing Hormone (GnRH) Agonists and Metabolites: Monitoring Adherence to Pharmacological Treatment for Libido Suppression	Nam Hee Kwon
P-121	Validation of a High-Resolution Mass Spectrometry (LC-HRMS/MS) Method for the Selective Detection and Quantitation of Gamma-Hydroxybutyrate (GHB) in Antemortem Urine	Carol Wood
P-122	Identification of a Unique Thermal Artifact at m/z 134.09477 as a Forensic Marker for Catha edulis Using DART-ToF-MS	Abdulrhman Dhabbah
P-123	Use of a New Derivatizing Method to Quantify Phosphatidylethanol by Gas Chromatography-Electron Impact-Mass Spectrometry	James DeFrancesco
P-124	Chronological Prevalence of Δ9-THC-COOH and Δ9-THCV-COOH from 1999–2020	Waseem Gul

POSTER SESSION 1 TUESDAY, SEPTEMBER 22 | 12:00-1:30PM | SALON LOBBY MODERATORS: ALICE SEYWRIGHT & MARISOL CASTANETO		
#	**TITLE**	**PRESENTER**
P-125	Retrospective Review of Mitragynine-Positive Casework in Dallas County for Mitragynine Pseudoindoxyl	Alain Peralt
P-126	Lactate-Dependent Tau K243 Lactylation Deficiency Impairs Hippocampal Mitochondrial Function Mediating Ketamine-Induced Neurotoxicity and Cognitive Impairment	Peipei Wang
P-127	Presence of Novel Psychoactive Substances in Negative Confirmed Benzodiazepine Clinical Urine Specimens	Nancy Yeomans
P-128	Cross-Reactivity of Novel Psychoactive Substances Using Immunoassay and Drug Screen Urine Collection Devices	Theresa Meli
P-129	Synthesis of 25E-NBOH Metabolites and Their Identification in Biotransformation Models	Petr Palivec
P-130	Metabolite Identification of a Difluorinated MDMA Derivative (DFMDMA) Using Human Liver Microsomes via UHPLC-HRMS/MS	Monika Mrňavá

POSTER SESSION 1 TUESDAY, SEPTEMBER 22 | 12:00-1:30PM | SALON LOBBY MODERATORS: ALICE SEYWRIGHT & MARISOL CASTANETO		
#	**TITLE**	**PRESENTER**
P-131	Use of Retrospective TOF Data-Mining to Assess Changes in Kratom Alkaloids in Authentic Toxicology Casework	Gabrielle Sawick
P-132	Evaluation of Three SelectraCore® HPLC Columns for the Separation of Orphine Analogues	Ritesh Pandya
P-133	Strategic Prevention and Reduction of Drug Misuse Using Knowledge Graphs (SPAR-KG): A Knowledge Graph Dashboard for Detecting Emerging Drug Trends in Social Media	Lynn Wagner
P-134	Drug Trends in North Louisiana: A Five-Year Review	Emily Raley
P-135	Hidden Administration of 5F-ADB, a Synthetic Cannabinoid, to Minors: Investigation in Blood, Urine, and E-Liquids	Frederic Aknouche
P-136	Analyses of LSD Analogs in Blotter Paper Products Available Online in Japan: Identification of 1EBP-LSD, 1PS-LSD, and 1Fe-LSD	Rie Tanaka
P-137	The Rise of Etomidate: Emerging Trends of Veterinary Sedatives and Nitazene Analogues from a National Wastewater Monitoring Campaign in Australia	Emma Barber

POSTER SESSION 1 TUESDAY, SEPTEMBER 22 | 12:00-1:30PM | SALON LOBBY MODERATORS: ALICE SEYWRIGHT & MARISOL CASTANETO		
#	**TITLE**	**PRESENTER**
P-138	Understanding Polydrug Use Patterns Using Networks and Machine Learning Techniques	Ankita Mondal
P-139	High-Dimensional Modelling of Forensic Time-Resolved Toxicology Data Using Random Forests and Neural Networks to Infer Cannabinoid-Associated Risk Behaviors	Paula Proença
P-140	Evaluation of an Integrated LC–MS Quantitation Workflow for Forensic Toxicology Applications	Victoria Starkie
P-141	Retro-BAC: An R Shiny Application for Retrograde Extrapolation Alcohol Calculation with IA Chatbot	José Moreno Paz
P-142	Postmortem Toxicology Trends in Pediatric Decedents Aged 6 Years and Under: A Retrospective STORM Surveillance Analysis	Ivan Padilla
P-143	Systemic Distribution of Tolfenpyrad and Its Major Metabolite PTCA in a Case of Fatal Poisoning	Natsuki Ikematsu

POSTER SESSION 1 TUESDAY, SEPTEMBER 22 | 12:00-1:30PM | SALON LOBBY MODERATORS: ALICE SEYWRIGHT & MARISOL CASTANETO		
#	**TITLE**	**PRESENTER**
P-144	Validated LC–MS/MS Quantification and Assessment of Postmortem Redistribution of Mirogabalin in a Fatal Multidrug Intoxication Case	Kenta Yuyama
P-146	Perimortem-Postmortem Drug Concentration Discrepancies in a Fatal Pentobarbital Intoxication	Sara Casati
P-147	Illicit Drug-Related Deaths in Singapore	Baohui Fu
P-148	Atypical Fatality in Postpartum Depression: Fatal Synergism of Alcohols, CNS Depressants, and Dissociative Anesthetic	Ansa Naeem
P-149	Determination of Hypophosphite in Blood by LC-MS/MS for the Confirmation of Phosphine Poisoning	Adrian Taylor
P-150	A Tale of Two Barbs: Suicidal Ingestion of Barbiturates in Two Unique Cases in Miami, FL	M Elizabeth Zaney

POSTER SESSION 1 TUESDAY, SEPTEMBER 22 | 12:00-1:30PM | SALON LOBBY MODERATORS: ALICE SEYWRIGHT & MARISOL CASTANETO		
#	**TITLE**	**PRESENTER**
P-151	Ethanol Distribution Across Blood, Vitreous Humor, and Urine: Associations with Manner of Death and Physiological State	Nelida Cristina Rubio
P-152	Postmortem Drug and Alcohol Detection Among Deceased Foreign Nationals in Bangkok, Thailand (2024–2025)	Jakkapan Boonsritan
P-153	Thailand's Poly-Drug Challenge: Kratom Co-Ingestion in Accidental vs. Suicidal Fatalities (2019–2024)	Apinya Tubtimrattana
P-154	Fatality Due to Combined Toxicity of Guaifenesin, Methorphan, and Fluoxetine	Anna T Kelly
P-155	A Study of Deaths Associated with Polysubstance Use at the Central Institute of Forensic Science, Thailand (2022–2025)	Tanasiri Yokchue
P-156	Use of Randox Biochip Array Technology and Correlation to Confirmatory 'Gold Standard' Techniques for Comprehensive Drug Screening	Victoria Anderson
P-157	Alignment Between DRE-Observed Indicators of Impairment and Toxicology Data in Polysubstance Use Cases	Kalila Daveron

POSTER SESSION 1 TUESDAY, SEPTEMBER 22 | 12:00-1:30PM | SALON LOBBY MODERATORS: ALICE SEYWRIGHT & MARISOL CASTANETO		
#	**TITLE**	**PRESENTER**
P-158	Development of a Portable Capillary Electrophoresis for On-Site Analysis of Illicit Drugs in Oral Fluid	Saari Anete Loog
P-159	Results Following a Two-Year Drug-Impaired Driving Backlog Project for the State of Texas	Sarah Martin
P-160	Characteristics of Alcohol- and Drug-Impaired Driving Reoffenders in Houston, 2019–2025	Corissa Rodgers
P-161	How Long It Takes to Obtain a DUI Blood Draw in Wyoming and Other DUI Statistics from the Cowboy State	Rachael Chavez
P-162	Assessment of Tissue Homogenization Methods for NPS Benzodiazepine and Fentanyl Analogues	Elizabeth Greco
P-163	Withdrawn	
P-164	Formalin-Fixed Paraffin-Embedded Tissue: A Suitable Matrix for Drug Screening?	Julius Hoffmann
P-165	The Bones Never Rest: Determination of Alkaloids in Bones in an Archeo-Toxicological Context	Geneva Loomis

POSTER SESSION 1 TUESDAY, SEPTEMBER 22 | 12:00-1:30PM | SALON LOBBY MODERATORS: ALICE SEYWRIGHT & MARISOL CASTANETO		
#	**TITLE**	**PRESENTER**
P-166	LC-MS/MS Detection of Cannabinoid Metabolites in Umbilical Cord Tissue for Assessment of Prenatal Cannabis Exposure	Wen Dui
P-167	Stability of Methamphetamine Concentrations in Vitreous Humor Across Prolonged Postmortem Intervals	Ayana Kishimoto
P-168	Identifying Glucuronide Substrate Biases in β-Glucuronidases for Toxicology Applications	William Murphy
P-169	Withdrawn	
P-170	The Analysis of Microbial Contamination in Electronic Cigarette Liquids and Generated Aerosols	Ankita Gola
P-171	Clinical Characteristics and Identified Drugs of Abuse Among People Who Use Drugs Admitted to a Tertiary Hospital in the Philippines Using a Validated LC-QTOF/MS Method	Ailyn M Yabes

POSTER SESSION 1 TUESDAY, SEPTEMBER 22 | 12:00-1:30PM | SALON LOBBY MODERATORS: ALICE SEYWRIGHT & MARISOL CASTANETO		
#	**TITLE**	**PRESENTER**
P-172	Quantification of 2,5-Hexanedione in Urine Using Liquid Chromatography Coupled with Tandem Mass Spectrometry	Szu-Yin Hsieh
P-173	Withdrawn	
P-174	Assessing the Prevalence of p-Phenylenediamine and Heavy Metals in Henna Tattoo Inks Purchased in Northern Delaware	Laell Sands
P-175	Surveillance of Novel and Multi-Chamber Vaping Devices	Kevin Lester
P-176	Sodium Nitrite Toxicity: Development of a Method for Rapid Toxicological Screening Using a Mobile Color-Detecting Application	Pichapar O-chongpian
P-177	Effects of Cigarette Smoking on Direct Alcohol Biomarkers Across Biological Matrices After Controlled Alcohol Administration	Asli Atasoy Aydin
P-178	Effects of Cyclodextrins on Cannabidiol Solubility and Stability in Simulated Gastric Fluid	Yuki Shimizu
P-179	Evaluating Synthetic Melanins for Benzodiazepine Uptake: Potential Forensic Applications	Valentina Martini

POSTER SESSION 1 TUESDAY, SEPTEMBER 22 | 12:00-1:30PM | SALON LOBBY MODERATORS: ALICE SEYWRIGHT & MARISOL CASTANETO		
#	**TITLE**	**PRESENTER**
P-180	Investigation of the Thermal Conversion of Cannabidiol (CBD) to Δ9-Tetrahydrocannabinol (THC) in CBD-Containing Smoking Products Using Pyrolysis-GC/QTOF-MS	Andy Koh
P-181	Validation of the Rudolph AutoFlex® R827 for Automated Human Urine pH and Specific Gravity Testing	Michael Chen
P-182	Identification of a UGT2B7 Residue Which Determines Glucuronidation at the 6-OH Position of Morphine, and Species Differences in This Residue and Morphine Glucuronidation	Yuji Ishii
P-183	Evaluating Post-Analytical Stability of Blood Ethanol Samples Using Headspace-GC-FID	David Cook

POSTER SESSION 2 WEDNESDAY, SEPTEMBER 23 | 12:00-1:30PM | SALON LOBBY MODERATORS: HOOI YAN MOY & GARRY BRENT DAWSON		
#	**TITLE**	**PRESENTER**
P-200	THC Isomers & Derivatives & Homologs, Oh My! – A Chromatographic Interference Study by UPLC-MS/MS Involving 45 Tetrahydrocannabinol Analogs	Courtney Meehan
P-201	Development of a Quantitative Method for Opioids in Blood Using LC-MS/MS and Filter Vials	Alexus Ramirez-Wiggins
P-202	Evaluating DART-QQQ-MS for Comprehensive Forensic Toxicology Sample Screening	Isabella Buttacavoli
P-203	Immunoassay for the Detection of Zolpidem in Urine on the Beckman Coulter AU480 Clinical Analyzer	Tu-An Le
P-204	Redevelopment of a High-Specificity Homogeneous Immunoassay for Ethyl Glucuronide (EtG) at Dual Cutoffs	Chris Wang
P-205	From Trace to Truth-A.S.A.P.! Drug Detection Can't Wait: Unknown Drug Analysis Using the RADIANTM ASAP-MS	Wilsa J Raymonvil
P-206	From Method to Matrix: Analytical Method Development and Pre-Validation Recovery Optimization of Neurotransmitters for Bioanalysis on LC-QTOF-MS	Laerissa Reveil
P-207	Beyond the Usual Suspects: Comprehensive Cannabinoid Screening in Whole Blood	Lillie Thomas

POSTER SESSION 2 WEDNESDAY, SEPTEMBER 23 | 12:00-1:30PM | SALON LOBBY MODERATORS: HOOI YAN MOY & GARRY BRENT DAWSON		
#	**TITLE**	**PRESENTER**
P-208	Conventional Plasma vs. Dried Microsampled Plasma – Development and Validation of Two Analytical Methods for Adherence Monitoring of Cardiovascular Disease Drugs	Diana Kretschmer
P-209	Enantioselective Analysis of Amphetamines in Serum, Urine, Hair, and Dried Blood Spots	Sophia Wrbas
P-210	Performance Evaluation of a Multi-Analyte LC–MS/MS Method in Blood Over Four Years of Application in Routine Casework	Dimitra Florou
P-211	Analytical Challenges in Forensic Chemistry Identification of Emerging Peptide and Protein Substances in Seized Materials	Jakob Hansen
P-212	Simultaneous LC-MS/MS Determination of Anabolic Androgenic Steroids and Selective Estrogen Receptor Modulators in Postmortem Blood: Validation and Forensic Application	So-Hyun Kim
P-214	Rapid Workflows for Triple Quadrupole LC-MS/MS Screening	Sarah Monti
P-215	Development of Monoclonal Antibodies Targeting Kratom Alkaloids and Nitazene Opioids: From Hapten Design to Immunochemical Applications	Martin Kuchar

POSTER SESSION 2 WEDNESDAY, SEPTEMBER 23 | 12:00-1:30PM | SALON LOBBY MODERATORS: HOOI YAN MOY & GARRY BRENT DAWSON		
#	**TITLE**	**PRESENTER**
P-216	Are You Stable? A Working Stock Stability Study	Mahagani Thomas
P-217	Metabolite Masquerade: False-Positive Norfentanyl in the Wake of Local Anesthetics	Elizabeth Taylor
P-218	Development and Validation of an Analytical Workflow for the Analysis of Psychedelics in Whole Blood	Rebecca Wagner
P-219	Re-Evaluation of Sample Preparation for Extraction Efficiency Improvement	Erin Cosme
P-220	Column Lifetime Performance in LC–MS/MS Pain Management Drug Testing: A Comparative Study	Stephanie J Marin
P-221	Performance Evaluation of the ARK Xylazine Assay on the Siemens Atellica DT 250 Analyzer	Rajendra Singh
P-222	Three Approaches, One Target: A Comparative Study of LC-MS/MS, LC-Q/TOF-MS, and ELISA for PCP, Ketamine, and Selected NPS Analogs	Andrea Bernal
P-223	Performance Evaluation of Second-Generation ARK Urine Drug Testing Assays on the Siemens Atellica DT 250 Analyzer	Thomas Houts
P-224	In Vitro Metabolism Comparison of Ortho-, Meta-, and Para-Methylfentanyl Using Recombinant CYP450 Enzymes	Fiza Tajdin
P-225	Evaluation of Long-Term Nitazene Stability in Preserved Whole Blood Stored at Different Temperatures	Sara Kuberski

POSTER SESSION 2 WEDNESDAY, SEPTEMBER 23 | 12:00-1:30PM | SALON LOBBY MODERATORS: HOOI YAN MOY & GARRY BRENT DAWSON		
#	**TITLE**	**PRESENTER**
P-226	In Vitro Phase I Metabolic Profiling of Synthetic Cathinones 3-MMC, N-Isopropyl Butylone, N-Cyclohexyl Methylone, and Alpha-PiHP	John Beer
P-227	Legal Responses to New Psychoactive Substances: Impact of the REITOX Network and Different Regulatory Approaches in Europe	Valeria Aquilina
P-228	Withdrawn	
P-229	Monitoring the Shift in Québec's New Psychoactive Substances (NPS) Detections: Findings from 2019–2025	Catherine Daigle
P-230	Retrospective Time-of-Flight (ToF) Screening of Forensic Toxicology Casework to Identify Novel Psychoactive Substances in Alabama	Katherine Smeltz
P-231	Mistaken Identity: A Prevalence Study of Bromazolam in the American Great Lakes Region	Chris Thomas
P-232	Prevalence and Toxicological Significance of 3-CMC and 4-CMC: A Retrospective Study of Over 250 Forensic Cases (2011–2026)	Piotr Adamowicz
P-233	Positional and Enantioselective Discrimination of Methylmethcathinones (MMC): LC–MS/MS Analysis of 2-, 3- and 4-MMC in Whole Blood	Telmo Gomes

POSTER SESSION 2 WEDNESDAY, SEPTEMBER 23 | 12:00-1:30PM | SALON LOBBY MODERATORS: HOOI YAN MOY & GARRY BRENT DAWSON		
#	**TITLE**	**PRESENTER**
P-235	Integrated Multi-Analytical Approaches for the Detection of New Psychoactive Substances: Identification of a New Brominated Synthetic Cannabinoid in Herbal Seizure Samples	Isadora Denkena
P-236	Di-Protonation-Driven Structural Elucidation of 5-Methyl Etodesnitazene Metabolites by LC-ESI± HRMS/MS	Anastasio Tini
P-237	Bridging Biosensing and Gold-Standard Analytics: Preliminary Analytical Evaluation of a MIP-QCM Platform for Fentanyl and Norfentanyl Detection in Human Urine	Dilek Battal
P-238	Development of a Molecularly Imprinted Polymer (MIP)-Based Quartz Crystal Microbalance (QCM) Biosensor for Detection of Alcohol Biomarkers (EtG/EtS) in Urine	F Defne Yaldiz
P-239	Evaluation of a Novel Oral Fluid Collection Device: Drug Recovery, Matrix Effects, and Analyte Stability	Qian Cheng
P-240	Withdrawn	
P-241	Now You See It, Now You Don't: The Rise, Fall, and Return of Para-Fluorofentanyl	Jade King
P-242	Assessment of Postmortem Urine Fentanyl Detection by Autopsy Dipstick Testing in Accidental Overdose Deaths	Anson Tsang
P-243	Death with Dipyanone: A Postmortem Polydrug Case in Montana	Crystal Everett

POSTER SESSION 2 WEDNESDAY, SEPTEMBER 23 | 12:00-1:30PM | SALON LOBBY MODERATORS: HOOI YAN MOY & GARRY BRENT DAWSON		
#	**TITLE**	**PRESENTER**
P-244	Metformin-Related Deaths Are Naturally Complicated	Katherine Ryding
P-245	Fatal Fentanyl-Associated Mixed-Drug Intoxication in a 46-Year-Old Woman with Psychiatric Comorbidities: Illicit Fentanyl Exposure in the Context of Prescribed Psychiatric Polypharmacy	Freddie John Ortiz-Colon
P-246	Prevalence and Forensic Interpretation of Toxicology Findings in Pediatric Mortality	Lisa Godsey
P-247	Evaluation of the Impact of Methamphetamine-Induced Rhabdomyolysis on Postmortem Redistribution	Kanako Noritake
P-248	Quantitative Measurement of Carbon Monoxide Using the HemoCD Assay: Differential Correlations with %COHb in Human Blood and Myocardial Tissue	Jun Yoshida
P-249	Postmortem Brain Carbon Monoxide Concentrations in Two Fatal Carbon Monoxide Poisoning Cases with Decreased Blood COHb Saturation After Oxygen Therapy	Kazuya Mori
P-250	Still Present, Still Lethal: Methamphetamine-Positive Deaths in a Changing Drug Landscape	Paul Simmons
P-251	Withdrawn	
P-252	Summary Statistics of Post-Mortem Blood Concentrations of Pharmaceuticals and Metabolites: An Analysis of Portuguese Data Over a Two-Year Period	Paula Proença

POSTER SESSION 2 WEDNESDAY, SEPTEMBER 23 | 12:00-1:30PM | SALON LOBBY MODERATORS: HOOI YAN MOY & GARRY BRENT DAWSON		
#	**TITLE**	**PRESENTER**
P-253	Cocaine and Death Due to Aortic Dissection: A Case Report	José Moreno Paz
P-254	Fatal Intoxication Involving the Novel Synthetic Opioids SR-17018 and Methiodone: A Case Report	Sabine Peisert
P-255	Testing Validity of the Standardized Field Sobriety Test (SFST) to Detect Presence of and Impairment by Cannabis and/or Alcohol: A Randomized Clinical Trial	Christine Wickens
P-256	Crabs, Old Bay, and Controlled Substances: A Ten-Year Retrospective Analysis of Impaired Maryland Drivers	Lauren Wolfe
P-257	Roadside Surveys of Drinking and Driving in Cameroon	Yannick Oyono
P-258	Unbiased Screening of DUID Cases for Gamma-Hydroxybutyric Acid (GHB)	Simone Tchu
P-259	Five-Year Retrospective Analysis of Alcohol and Drug Involvement in Motor Vehicle-Related Fatalities in Jefferson County, Alabama	Hannah Hynds
P-260	Drug Detection Trends in Umbilical Cord and Meconium and the Impact of Enzymatic Hydrolysis in Cord Analysis	Tiara Evans
P-261	Exploring Human Bone Specimens as an Alternative Biological Matrix for Drug Screening Using Gas Chromatography-Mass Spectrometry (GC-MS): Potentialities and Limitations	Paul Jimenez

POSTER SESSION 2 WEDNESDAY, SEPTEMBER 23 | 12:00-1:30PM | SALON LOBBY MODERATORS: HOOI YAN MOY & GARRY BRENT DAWSON		
#	**TITLE**	**PRESENTER**
P-262	Evaluation of the Cross-Reactivity of the Narcotrace™ 8-Drug Panel Oral Fluid Device for Cannabinoid and Semi-Synthetic Cannabinoid Compounds	Elizabeth Southorn
P-263	Mimicking the Matrix: Early Development of a Synthetic Serum Blank for Toxicological Applications	Matthew Gregory
P-264	Extraction of NSAIDs from Horse Urine Using QuEChERS	Ritesh Pandya
P-265	Analyzing the Impact of Centrifugation and Extraction Aids on Oral Fluid Sample Recovery	Samantha Herbick
P-266	Case of Consistency: Oral Fluid as a Tool to Monitor Mental Health Medications in Clinical Settings	Matthew Levitas
P-267	Evaluation of THC Acid A as a Marker for External Contamination for the Analysis of Capillary Blood from the Finger Pulp	Joel Bruegger
P-268	Socioeconomic Stratification in Alcohol Consumption During the Pandemic: Evidence from Wastewater-Based Epidemiology and Predictive Decision Modelling	Nebile Daglioglu
P-269	Assessment of Second-Hand Nicotine Exposure via Cross-Sectional Biomonitoring	Ezgi Bezci
P-270	Rapid Enzymatic Hydrolysis for Fast Screening of New Psychoactive Substances in Urine Samples from Emergency Toxicology Cases	Rafael Lanaro

POSTER SESSION 2 WEDNESDAY, SEPTEMBER 23 | 12:00-1:30PM | SALON LOBBY MODERATORS: HOOI YAN MOY & GARRY BRENT DAWSON		
#	**TITLE**	**PRESENTER**
P-271	Terbufos Intoxication in Humans and Animals: Toxicokinetics and Analytical Findings from Two High-Profile Taiwanese Cases	Te-i Weng
P-272	Extraction of Tobacco-Specific Nitrosamines (TSNAs) from Human Urine Prior to UPLC-MS/MS Analysis	Sohel Rana
P-273	A Five-Year Comparison of Seized Drug and Toxicology Trends	Amy Patton
P-274	Toxicological Assessment of Fentanyl, Xylazine, and BTMPS Through Zebrafish Developmental Model	Danielle Malenius
P-275	Opioid–Stimulant Co-Use in San Francisco: Trends in Overdose Mortality and Human Performance Toxicology	Jessica Winborn
P-276	Analytical Findings from a Consumer Case Involving a Triple-Chamber Cannabinoid Vape	Jessemia Meekins
P-277	A Heuristic Evaluation of Point-of-Care Urine Tetrahydrocannabinol (THC) Test Kits	Madeline Hardesty
P-278	The Prevalence of Drug Poisoning and Overdose in Riyadh, Saudi Arabia	Jaber Almutairi
P-279	Analysis of Confiscated Vaping Products from Public Schools Across the Commonwealth of Virginia During the 2025–26 Academic Year	Meredith Buckmire
P-280	Trends in Multidrug Use Patterns: A 10-Year Comprehensive Analysis of Urine Drug Testing Reports	Serap Akgür

POSTER SESSION 3 THURSDAY, SEPTEMBER 24 | 12:00-1:30PM | SALON LOBBY MODERATORS: MIRIAM BLANCO-CES & DOMINIQUE GIDRON		
#	**TITLE**	**PRESENTER**
P-300	Direct Determination of Methamphetamine and Amphetamine in Urine by Thermal Desorption Counter-Flow Introduction Atmospheric Pressure Chemical Ionization Mass Spectrometry	Hiroyuki Inoue
P-302	Evaluation of the Stability and Cross-Reactivity of Zopiclone and Eszopiclone Using Immunoassay Kits	Takeshi Saito
P-303	Performance Evaluation of Antibody–Antigen Conjugate Pairs for Fentanyl and Norfentanyl Detection in Lateral Flow Assays	Mikael Jumppanen
P-304	Sensitive Quantification of Tetrodotoxin in Blood and Urine by LC-MS/MS	Adrian Taylor
P-305	Rapid LC–MS/MS Determination of 2C-Series Phenethylamines and NBOMe Analogues	Adrian Taylor
P-306	Withdrawn	
P-307	Development of a Highly Specific Competitive Immunoassay for the Quantification of Gabapentin in Human Blood Samples	Victoria Anderson
P-308	Evaluation of a New Multiplex Drugs of Abuse Immunoassay on the Evidence RABTA Analyser for Rapid Urine Testing	Victoria Anderson
P-309	Easy Strategy for the Addition of New NPS Compounds: Differentiation of Methylfentanyl Isomers and Isobaric Compounds	Eugénie-Raphaëlle Bérubé

POSTER SESSION 3 THURSDAY, SEPTEMBER 24 | 12:00-1:30PM | SALON LOBBY MODERATORS: MIRIAM BLANCO-CES & DOMINIQUE GIDRON		
#	**TITLE**	**PRESENTER**
P-310	Field-Based Quantitative Analysis of Cannabis Samples via Portable Near-Infrared Spectroscopy	Bronislav Jurásek
P-311	Assessment of Authentic Postmortem Vitreous Humor Biochemistry by Nova Prime Plus	Douglas Wong
P-312	Determination of Pregabalin in Biological Samples (Blood, Urine) by LC-MS/MS	Chang Jing
P-313	Withdrawn	
P-314	The Use of a Novel Quadrupole Time-of-Flight Instrument for the Quantitative and Sensitive Analysis of Drugs of Abuse in Hair Samples	Jonathan Danaceau
P-316	Assessment of Acquisition Strategies for Qualitative Drug Screening Using the SCIEX ZenoTOF 7600	Kendra Adams
P-317	A Robust and Automated LC–MS/MS Workflow for Quantitative Urine Drug Testing Across a Broad Dynamic Range on a Triple Quadrupole Mass Spectrometer	Holly Pagnotta
P-318	Practical Implementation of a Single-Platform Software Workflow for Forensic Toxicology Screening to Confirmation	Kala Babb
P-319	The Analysis of 12 Cannabinoids in Whole Blood by LC-MS/MS	Tim Fassette

POSTER SESSION 3 THURSDAY, SEPTEMBER 24 | 12:00-1:30PM | SALON LOBBY MODERATORS: MIRIAM BLANCO-CES & DOMINIQUE GIDRON		
#	**TITLE**	**PRESENTER**
P-320	A Case of Suspected Fentanyl Laced with Bromethalin Rodenticide	Andrea Edel
P-321	Withdrawn	
P-322	Understanding the Metabolism of Ethylbromazolam Using Human Liver Microsomes	Nathan Layle
P-323	Analysis of 7-Hydroxymitragynine and Mitragynine in Urine Workplace Drug Testing Specimens at CRL	Melissa Beals
P-324	First Detection of Four 2-Oxo Arylcyclohexylamine Analogues in Korea: Forensic Identification and Drug Trend Analysis	Jihyun Kang
P-325	Metabolic Evaluation of the Synthetic Opioid U-47700 Using In Vitro and In Vivo Approaches for Forensic Toxicology Applications	Beata Bystrowska
P-326	Forensic Detection of Nitazenes in Ontario: A Retrospective Analysis of Fatal Toxicity and Impaired Driving Cases (2019–2025)	Andrew Williams
P-327	Cardiotoxicity of Chloromethcathinones (CMC) in Postmortem Studies	Martyna Maciow-Glab
P-328	Withdrawn	
P-329	Synthesis and Analysis of Metabolites of Hexahydrocannabinol (HHC) and Its Analogs	Kei Ieuji

POSTER SESSION 3 THURSDAY, SEPTEMBER 24 | 12:00-1:30PM | SALON LOBBY MODERATORS: MIRIAM BLANCO-CES & DOMINIQUE GIDRON		
#	**TITLE**	**PRESENTER**
P-330	Miniaturized Salting-Out-Assisted Liquid-Liquid Extraction Coupled to LC-MS/MS for the Quantification of Nitazene Analogs in Plasma	Karla Aparecida de Oliveira Souza
P-331	Structure Characterization of Newly Emerging Cannabinoids: THC-P and HHC-P	Vladimír Setnička
P-332	Cytotoxic, Genotoxic, and Oxidative Stress-Mediated Toxicity of Synthetic Cathinones: Evidence from Experimental Models	Selda Mercan
P-333	Cytotoxic Effects of MDMB-4EN-PINACA and 5F-ADB in HepG2, SH-SY5Y, and AC16 Cell Lines	Selda Mercan
P-334	Is Xylazine Being Replaced by Medetomidine in Fentanyl Cases in Alabama?	Mary Ellen Mai
P-335	Carfentanil: You Don't Know What You Can't See. Are You Seeing the Whole Picture?	Rebecca Lin
P-336	From Metabolite to Misuse: O-Desmethyltramadol in Two Mixed-Drug Fatalities and Seized Materials	Maryline Busschots
P-337	A Five-Year Longitudinal Evaluation of the Behavioral and Toxicological Factors Defining the Trauma-Toxicity Continuum in Methamphetamine Fatalities	Torki Zughaibi
P-338	When Ecstasy Isn't So Ecstatic: A Possible Case of Suicide by MDMA	Jennifer Gonyea

POSTER SESSION 3 THURSDAY, SEPTEMBER 24 | 12:00-1:30PM | SALON LOBBY MODERATORS: MIRIAM BLANCO-CES & DOMINIQUE GIDRON		
#	**TITLE**	**PRESENTER**
P-339	Forensic Toxicological Interpretation of Fatal Overdose Involving Fentanyl and Multiple Co-Intoxicants	Luz Silva Torres
P-340	Drug-Involved Pediatric Deaths in Georgia: A Forensic Toxicology Perspective	Denise Carter
P-341	Low-Concentration Acetone as a Negative Indicator for Significantly Elevated BHB in Postmortem Casework	Glen Shoemaker
P-342	The Rise of Nitazenes: A Scottish Perspective	Alice Seywright
P-343	Screening, Confirmation, Prevalence, and Fatality of Digoxin in Finland in 2012–2025	Antti Jylhä
P-344	Fatal Mixed-Drug Intoxication Involving Methadone, Fluoxetine, Tramadol, and Multiple Sedative Co-Exposures	Reinalyz Alfonso Aragonés
P-345	Withdrawn	
P-346	Fatal Polydrug Intoxication in a Chemsex Context: Forensic Challenges in the Interpretation of Synthetic Cathinones	Marta Vázquez
P-347	LC-MS/MS Chiral Analysis of Ketamine in Danish Drug Seizures and DUID Blood Samples: Forensic Implications of Racemic Prevalence	Jakob Ross Jornil
P-348	Oral Fluid Versus Blood for Delta-9-Tetrahydrocannabinol and Cocaine: Concordance, Discordance, and Limits of Concentration Prediction	Franck Saint-Marcoux

POSTER SESSION 3 THURSDAY, SEPTEMBER 24 | 12:00-1:30PM | SALON LOBBY MODERATORS: MIRIAM BLANCO-CES & DOMINIQUE GIDRON		
#	**TITLE**	**PRESENTER**
P-349	Prevalence of Alcohol Among Drivers, Riders, and Pedestrians Injured in Road Traffic Crashes in Cameroon: A Cross-Sectional Study	Yannick Oyono
P-350	Drug-Facilitated Sexual Assault in Israel: A Retrospective Toxicological Study (2020–2025) Examining Post-Policy Change Trends	Anat Shriki
P-351	Establishment of a Medication Compliance Threshold for Probationers Ordered to Take Quetiapine, Risperidone, and Aripiprazole Using Urinalysis and ROC (Receiver Operating Characteristics) Analysis	Nam Hee Kwon
P-352	The Advantages of Fold Changes in Comparing THC Concentrations in Breath After Cannabis Use	Jennifer Berry
P-353	Accelerating Drug Screening in Forensic Hair Analysis by Applying High-Speed Polarity Switching in HR LC-MS/MS	Sarah Monti
P-354	Passive Cocaine Exposure Through Kissing or Sexual Contact	Pinar Efeoglu Ozseker
P-355	Detection of Fentanyl and Analogs in Oral Fluid by Homogeneous Enzyme Immunoassay	Warren C Rodrigues
P-356	Concentration Distributions and Metabolite-to-Parent Ratios in Hair Toxicology: A Retrospective Study	Ayako Chan-Hosokawa
P-357	Optimised Sample Preparation for Drugs of Abuse Extraction From Oral Fluid Prior to UHPLC-MS/MS Analysis	Russell Parry

POSTER SESSION 3 THURSDAY, SEPTEMBER 24 | 12:00-1:30PM | SALON LOBBY MODERATORS: MIRIAM BLANCO-CES & DOMINIQUE GIDRON		
#	**TITLE**	**PRESENTER**
P-358	Optimised Sample Preparation for Cannabinoids Extraction From Oral Fluid Prior to UHPLC-MS/MS Analysis	Russell Parry
P-359	ALDH2 Deficiency Exacerbates APAP-Induced Liver Injury via the ALOX12–12-HETE Axis	Weihao Fan
P-360	Withdrawn	
P-361	Impact of Environmentally Induced Hypothermia on Fentanyl and Norfentanyl Pharmacokinetics Following Intravenous Administration to Wistar Rats	Sebastian Rojek
P-362	Methanol Poisoning Outbreak in Brazil: A Case Series and the Role of a Regional Poison Control Center in Clinical and Analytical Management	José Luiz Costa
P-363	PEth Recovery Pilot Study: The Effects of Time and Temperature on Cellular Localization of PEth	Carl E Wolf
P-364	Withdrawn	
P-365	Changing Patterns of Illicit Drug Seizures in South Korea in 2025: Expansion of Ketamine, Phencyclidine Analogues, and Anesthetic-Related Substances	Hyeyoung Choi

POSTER SESSION 3 THURSDAY, SEPTEMBER 24 | 12:00-1:30PM | SALON LOBBY MODERATORS: MIRIAM BLANCO-CES & DOMINIQUE GIDRON		
#	**TITLE**	**PRESENTER**
P-366	False Positives as Barriers to Justice: Evaluating the Reliability of Presumptive Drug Tests	Leon Lippincott
P-367	Extracellular Particle Dynamics and Lipidomic Alterations Following Nandrolone Administration: Toward Novel Biomarkers for Anti-Doping	Sayaka Nagasawa
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O-001

50-Year Retrospective and Appreciation: Journal of Analytical Toxicology (JAT)

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Abstract

Now in its 50th year, the Journal of Analytical Toxicology (JAT) stands as a cornerstone of forensic and clinical toxicology, tracing a trajectory of scientific rigor, innovation, and community service since its inaugural issue in 1977 under founding Editor-in-Chief Dr. Randall C. Baselt. Throughout its evolution from PubMed/MEDLINE indexing beginning in 1980, early adoption of online publishing in 1996, first tracked Impact Factor in 1997, and the 2011 acquisition by Oxford University Press, JAT has continually advanced the field's analytical standards and knowledge base and provides valuable continuing education and communication platforms for toxicologists, partnering professionals, and the public.

By 2026, JAT has transitioned to a fully online format and publishes nine issues annually, reflecting growing global demand for high-quality toxicology research. As the official journal of both the Society of Forensic Toxicologists (SOFT) and The International Association of Forensic Toxicologists (TIAFT), JAT has supported scientific exchange across continents, producing more than 4,000 peer-reviewed articles read in over 100 countries. Its most-cited works including the SWGTOX standard practices for method validation, landmark studies on drug pharmacokinetics, postmortem, drug-facilitated sexual assault, and impaired driving casework, and innovative methodologies demonstrate how JAT has shaped both laboratory practice and public health policy.

The journal's success is rooted in the dedication of its editorial leaders, from Dr. Baselt's foundational vision to Dr. Bruce A. Goldberger's decades of stewardship and now continue with our commitment to build on their legacy, supported by the exceptional work of our managing editor and publisher. JAT further owes its strength to the thousands of authors, reviewers, editorial board members, associate and guest editors, and the broader SOFT and TIAFT communities whose expertise and service have defined and advanced its scientific foundation. Their contributions support professional recognition such as the Editor's Choice Articles and the SOFT's EDIT Award, reinforcing JAT's role in spotlighting excellence in toxicology research.

Together, these milestones and contributions illustrate JAT's enduring commitment to advancing forensic and analytical toxicology. The journal's 50th anniversary special collection, now available online, honors all the invaluable contributors who have influenced its growth and impact. As JAT celebrates its 50-year anniversary, coinciding with the 2026 SOFT-TIAFT joint meeting, it remains steadfast in its mission to publish impactful, relevant, and scientifically rigorous research that informs and elevates toxicology practice worldwide.

Disclosure

Yes, I, or a member of my immediate family, has a financial interest to disclose.

Conflict of Interest

Active contract with Oxford University Press as the co-editor-in-chief of the Journal of Analytical Toxicology

O-002

From Method to Mainstay: One Year of LC-QTOF-MS

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Abstract

Introduction: The rapid emergence of novel psychoactive substances (NPS) and increasing complexity of forensic toxicology casework have accelerated the transition from traditional screening to liquid chromatography–mass spectrometry (LC-MS) platforms. High-resolution mass spectrometry (HRMS), particularly liquid chromatography quadrupole time-of-flight mass spectrometry (LC-QTOF-MS), enables accurate-mass screening, retrospective data analysis, and broad compound coverage. However, operational evaluations of HRMS implementation in routine, high-volume forensic laboratories remain limited. This study describes the practical integration of an LC-QTOF-MS method with existing workflows at the San Francisco Office of the Chief Medical Examiner (OCME) and evaluates its impact on casework efficiency, analytical scope, and workflow.

Objectives: This study aimed to evaluate the operational impact of integrating LC-QTOF-MS into routine forensic toxicology workflows, assess improvements in laboratory efficiency and turnaround time, characterize the expansion of analytical scope and detection of emerging drugs, and examine the role of automated data processing, LIMS integration, and retrospective analysis in supporting high-volume casework.

Methods: A validated LC-QTOF-MS method encompassing 946 analytes across 35 drug classes was implemented using SWATH acquisition on a SCIEX X500R instrument. The workflow was integrated with existing LC-MS-MS methods used for quantitative confirmation. Routine casework involved protein precipitation and filtration prior to analysis. Automated data processing was incorporated to streamline batch review and reporting. One year of routine casework data was evaluated following implementation. Metrics assessed included turnaround time, detection frequency, analytical coverage, and agreement between semi-quantitative QTOF estimates and LC-MS-MS quantitative results. Library updates and retrospective data mining were also assessed.

Results: The integrated workflow substantially improved operational efficiency. Automated data processing reduced batch review time from approximately two days to three hours, representing a 93.75% reduction in analyst review time. Across the study period, over 20,000 detections were confirmed, including confirmations by duplicate QTOF analysis or metabolite identification. The workflow demonstrated an overall efficiency of 99%, sensitivity of 99%, and specificity of 99%. LC-QTOF-MS identified over 400 detections not captured by LC-MS-MS methods, with over 300 unique analytes detected in casework, including multiple emerging NPS and uncommon pharmaceuticals. Importantly, over half of the unique detected components were outside the scope of existing LC-MS-MS methods and would have previously gone undetected. Semi-quantitative QTOF estimates aligned with LC-MS-MS quantitative values within method uncertainty for 57% of comparisons. Quarterly library updates added 40 compounds, and retrospective data analysis identified additional detections of newly incorporated analytes.

Discussion: Implementation of LC-QTOF-MS within a routine forensic toxicology workflow significantly improved analytical breadth and operational efficiency while maintaining reliable reporting timelines. Integrating screening and confirmation within a unified LC-QTOF-MS and LC-MS-MS workflow reduced reliance on sequential assays and enabled more comprehensive case interpretation. Automated data processing and LIMS integration further streamlined batch review and improved data traceability. The expanded analytical scope allowed detection of emerging drugs and uncommon substances not included in targeted assays, strengthening both medicolegal interpretation and public health surveillance. Additionally, retrospective analysis enabled identification of compounds added to the library after initial analysis, demonstrating the long-term value of HRMS datasets. Overall, these findings illustrate how LC-QTOF-MS can be sustainably deployed in high-volume forensic laboratories to meet evolving toxicological challenges while improving workflow efficiency and analytical capability.

Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

O-003

Optimizing Sample Extraction and Data Review of LC-QTOF/MS All Ions Screening Using Microsoft Excel

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Abstract

Introduction: Comprehensive drug screening by liquid chromatography-quadrupole time-of-flight mass spectrometry (LC-QTOF/MS) with All Ions acquisition has become increasingly valuable in forensic toxicology. By enabling non-discriminatory collection of accurate-mass and fragment ion information, this approach broadens analytical scope while preserving full-scan data for retrospective interrogation. Despite this major advantage, routine implementation is limited by the complexity of data review, particularly when compatible vendor software or built-in tools for streamlined interpretation are lacking. Under these conditions, presumptive findings must often be manually compared against quality controls across multiple decision criteria (e.g., chromatographic quality, analytical response, mass error, retention time difference, library match score) in a consistent and efficient manner. As case complexity grows, this repeated manual assessment becomes increasingly labor-intensive, time-consuming, and more susceptible to human error and analyst fatigue. Microsoft Excel is a ubiquitous tool that provides a practical platform for organizing large datasets and supporting a standardized review process.

Objectives: Develop an integrated sample extraction protocol and Microsoft Excel macro-based review workflow to facilitate interpretation of Agilent LC-QTOF/MS All Ions screening data.

Methods: Analyte recovery and matrix effects were evaluated utilizing post-extraction addition to compare multiple sample preparation approaches. These approaches included protein precipitation followed by filtration with UCT FASt®, UCT Refine®, Agilent Captiva, Phenomenex Phree phospholipid removal columns, or Thomson PTFE filter vials, in addition to supported liquid extraction using Biotage ISOLUTE SLE+.

All blood samples were analyzed using an Agilent 1290 Infinity II Liquid Chromatograph coupled to Agilent 6545 QTOF/MS. Data were processed using a target-suspect screening workflow in Agilent MassHunter Qualitative Analysis version 10. Library matches generated using a personal compound database and library were exported from “Compounds View” and directly integrated with the customized spreadsheet. This Microsoft Excel workflow was developed using simple formulas, including VLOOKUP and IF functions, to apply identification criteria consistent with ANSI/ASB 113 in a structured review format.

Results: Acceptable recovery and matrix effects were generally observed across the 173 drug panel using protein precipitation followed by UCT Refine® filtration.

Decision criteria incorporated accurate mass error within ±5 ppm, retention time within ±0.1 minutes of a concurrently analyzed positive control, library match score >70, and peak area ratio of the sample relative to the positive control at predefined analytical thresholds with guidance according to ANSI/ASB 119. Based on these criteria, compounds were assigned a “Pass,” “Fail,” or “Additional Review” result in the final review table, depending on whether all, none, or some of

the criteria were met. These categories allowed analysts to filter the results and efficiently triage presumptive identifications. Embedded instructions guided analysts through review of peak area ratios, presence of fragment ions, and chromatographic quality, based on the complementary report. Altogether, these combined outputs supported consistent review of presumptive identifications and selection of compounds for downstream confirmation across analysts, analytical runs, and compound classes.

Discussion: This approach combines efficient sample preparation, All Ions acquisition, and an Excel-based review framework to support practical LC-QTOF/MS implementation in routine forensic toxicology. This workflow improves consistency, supports traceability when prioritizing samples for confirmation, and provides an accessible review structure for laboratories using Agilent instrumentation.

Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

O-004

Integration of an All Ions HRMS and LC Screener Strategy for Detection of Emerging and Prevalent Psychoactive Compounds

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Abstract

Introduction: High-resolution mass spectrometry (HRMS) has become an increasingly critical tool in modern forensic toxicology, yet data-processing demands are often cited as a barrier to routine implementation. At the Alabama Department of Forensic Sciences, HRMS data have traditionally been processed using Agilent Qualitative Analysis; however, this approach can be time-intensive for larger batches. To address this limitation, we evaluated the use of Agilent's LC Screener workflow found within MassHunter Quantitative Analysis software as an alternative processing strategy. In parallel, heightened analytical sensitivity is essential for monitoring novel psychoactive substances (NPS) due to their potency. Accordingly, this study also investigated an All Ions HRMS method detection of three emerging regional NPS and three established dissociative-class compounds.

Objectives: This study aimed to: (1) develop an HRMS method using All Ions data acquisition; (2) establish a rapid data-analysis workflow using the LC Screener tool; and (3) apply this combined approach to a limit of detection (LOD) evaluation for six psychoactive substances of interest.

Methods: An analytical method was implemented on an Agilent 6545 Q-TOF LC/MS system equipped with an Agilent Poroshell 120 EC-C18 (2.1 × 100 mm, 2.7 µm) column. Specimens underwent protein precipitation with acetonitrile followed by multi-layer filtration and nitrogen dry-down. Samples were reconstituted and analyzed using All Ions data-independent acquisition at 0, 20, and 40 eV.

A complementary data-analysis workflow was developed in MassHunter Quantitative Analysis 12 using the LC Screener module. An in-house spectral library containing retention times and reference spectra for the target analytes was imported. Acceptance criteria were optimized to include a 0.1-min retention-time window, 10-ppm mass-accuracy tolerance, minimum signal-to-noise ratio of 3, ion co-elution score ≥80, and confirmation by at least two diagnostic ions.

For LOD assessment, analytes were fortified into bovine blood in triplicate across three separate days and evaluated from 2 to 200 ng/mL. The LOD was defined as the lowest concentration at which ≥75% of replicates satisfied all screening criteria without being flagged as outliers by the LC Screener tool.

Results: The analytical method successfully resolved the two isobaric mitragynine derivatives with over 0.5 min of chromatographic separation. Retention times demonstrated excellent reproducibility, with zero instances of the retention time difference exceeding the acceptance criteria. The All Ions acquisition approach provided high-quality spectral data, yielding mass accuracies within ±5 ppm for both precursor and fragment ions. Using the LC screener workflow, LOD values (ng/mL) were determined to be: 7-hydroxymitragynine (20), mitragynine pseudoindoxyl (10), N-propionitrile chlorphine (5), phencyclidine (20), ketamine (5), and norketamine (10).

Discussion: This study demonstrated the effective integration of a new analytical method with an optimized data-analysis workflow. LC Screener improved efficiency by enabling simultaneous searching of multiple libraries containing over 200 compounds and permitted analyte-specific integration criteria rather than the global settings of Qualitative Analysis. In Qualitative Analysis, reports are generated sequentially, whereas the LC Screener produced reports for the entire batch within one minute, saving hours of processing time. Together, these features support enhanced detection of emerging and prevalent psychoactive substances in the region.

Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

O-005

Multi-Platform GC-EI-HRMS/LC-ESI-HRMS Characterisation of Post-Mortem Vitreous Humour for Forensic Applications Using a Curated Open-Access GC-HRMS Library for GC-Based Annotation

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Abstract

Introduction: Thanatometabolomics, the analysis of endogenous small molecules in post-mortem biofluids, is an emerging analytical strategy in forensic science focused on identifying novel potential biomarkers to help address ongoing challenges such as post-mortem interval estimation and cause-of-death determination. In this field, vitreous humour presents itself as an interesting alternative matrix compared to blood, due to its metabolically stable nature, arising from its protected location within the eye. To date, its metabolomic profiling is largely restricted to LC-HRMS in both clinical and forensic contexts, while GC-HRMS remains underexplored despite its proven value in environmental, food, and toxicological applications. Integrating both techniques is therefore essential to expand chemical coverage in vitreous humour. However, GC-HRMS identification is still strongly impeded by the scarcity of publicly available high-resolution spectral libraries, forcing current annotation strategies to rely heavily on low-resolution databases. This discrepancy between high-resolution acquisition and low-resolution libraries represents a key bottleneck limiting the full exploitation of the complementary advantages offered by GC-HRMS.

Objectives: An untargeted multi-platform approach integrating GC-EI-HRMS and LC-HRMS is developed and applied to comprehensively profile post-mortem vitreous humour for forensic applications, including the development of an open-access GC-HRMS library for GC-based annotation. The complementarity of both platforms in expanding metabolome coverage is evaluated.

Methods: For 90 authentic reference standards, 50 µg/mL solutions were prepared and derivatized using methoxyamine hydrochloride in pyridine, followed by N,O-bis(trimethylsilyl)trifluoroacetamide with 1% trimethylchlorosilane. Analyses were performed on an Agilent 8890 GC coupled to a 7250 EI-QTOF-MS. High-quality spectra were acquired using MS-DIAL and manually verified against the NIST library. Kovats retention indices were calculated, and metadata (formula, SMILES, RI, InChIKey) were appended to each spectrum in R, resulting in the compilation of an .msp library.

Vitreous humour samples were extracted using a previously optimized Bligh-Dyer protocol (chloroform:methanol:water, 2:2:1.8). Polar and non-polar fractions were evaporated under nitrogen and reconstituted for LC analysis or derivatized for GC analysis as described above. The reconstituted polar fraction was analysed using hydrophilic interaction liquid chromatography, while the non-polar fraction was analysed using reversed-phase liquid chromatography, both coupled to high-resolution QTOF-MS operated in positive and negative electrospray ionisation modes.

Data processing was conducted in MS-DIAL, including peak detection, deconvolution, alignment, and spectral matching. Signal drift correction, QC assessment, and feature filtering were performed in R using the Notame package. Metabolite annotation was based on both public databases and existing in-house LC-HRMS libraries as well as the curated GC-HRMS spectral library.

Results and Discussion: Combination of LC-HRMS and GC-HRMS confirmed the predominant presence of low-molecular-weight acidic compounds in vitreous humour, including amino acids, organic acids, and fatty acids. An open-access data-analysis workflow was developed, enabling implementation of the curated GC-HRMS library, which finally comprised 90 authentic standards. Approximately 60% of the GC-HRMS spectral matches could be assigned a level 1 identification based on this curated library. In addition, GC-HRMS expanded the metabolome coverage by substantially improving the detection of sugars and sugar derivatives compared to LC-based approaches. These results highlight the value of multi-platform approaches for broadening the endogenous metabolite coverage in post-mortem vitreous humour, which is essential for advancing biomarker discovery for forensic applications.

Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

O-006

Supercritical Fluid Chromatography Coupled to High-Resolution Mass Spectrometry: Application to the Detection of Drugs of Abuse in Oral Fluid

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Abstract

Introduction: Supercritical fluid chromatography (SFC) offers an attractive alternative to conventional liquid chromatography, using compressed CO_2 as the primary mobile phase. Owing to its low viscosity and high diffusivity, SFC enables rapid and efficient separations. Its tunable mobile-phase properties allow the analysis of compounds across a broad polarity range, while its excellent compatibility with mass spectrometry facilitates sensitive and selective detection.

Objectives: This study aimed to optimize chromatographic and spectral acquisition parameters for coupling SFC with high-resolution mass spectrometry (HRMS), and to apply this approach to the screening and quantification of drugs of abuse in oral fluid.

Methods: Amphetamine-type stimulants (methamphetamine, MDA, MDEA, MDMA, amphetamine), cocaine derivatives (benzoylecgonine, ecgonine methylester, cocaine) and opiates (6-acetylmorphine, morphine) were grouped as ACO analytes, while cannabinoids (THC, CBD) were considered separately. The analytical platform consisted of an SFC system coupled to a QTOF-HRMS instrument (SFC30A, LCMS9030, Shimadzu®). Oral fluid samples were prepared by protein precipitation using QuEChERS salts for ACO analytes, or by offline solid-phase extraction on Oasis HLB cartridges for cannabinoids. A design-of-experiments approach was used to select chromatographic columns, define supercritical elution conditions and compare targeted acquisition modes (SIM, MRM) with untargeted approaches (DIA/DDA, full-scan MS). The method was validated according to an internal procedure compliant with ISO 15189 requirements, assessing linearity, limits of detection and quantification, repeatability, reproducibility and accuracy. SFC-HRMS results were then compared with those obtained using the routine LC-MS/MS method in 120 oral fluid samples collected during roadside drug controls.

Results: Chromatographic separation was achieved on a Diol II column (Shimpack®) for ACO analytes, whereas a Chiralpak IG3 (Daicel®) column was required to separate THC and CBD isomers. For both columns, SFC conditions consisted of a temperature of 25°C and a pressure of 150 bar, using supercritical CO_2 as the main mobile phase and methanol containing 5 mM ammonium formate and 0.1% formic acid as co-solvent. A post-column methanol make-up flow of 0.1 mL/min was added to improve ionization. Elution was performed using a gradient from 2% to 40% co-solvent over 7 min at 1.3 mL/min. Spectral acquisition was performed in positive electrospray ionization mode using full-scan MS from 100 to 500 amu, followed by targeted MS/MS acquisition of the analytes and their deuterated internal standards. The method was validated over 5–500 ng/mL for ACO analytes and 0.5–100 ng/mL for cannabinoids, with limits of detection ranging from 0.5 to 2.5 ng/mL. Complete qualitative agreement between SFC-HRMS and LC-MS/MS, defined as concordance regarding the presence or absence of each analyte, was observed across the 120 real cases, including both positive and negative samples.

Discussion: This feasibility study demonstrates that SFC-HRMS enables sensitive detection of drugs of abuse in oral fluid, with complete qualitative agreement with the reference LC-MS/MS method. Beyond analytical performance, full-scan HRMS data offer significant potential for routine screening, targeted confirmation and retrospective data interrogation. By reducing organic solvent consumption by more than 90%, this approach represents a versatile and environmentally sustainable analytical platform for forensic toxicology.

Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

O-007

From Research to Routine - Evaluating Endogenous Biomarker's Performance Indicating Sample Storage Conditions of Blood Specimens in 489 Authentic Forensic Toxicology Samples

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Abstract

Introduction: Improper sample storage can compromise quantitative analyses and, consequently, forensic toxicological (FT) interpretation, as certain drugs, e.g., cocaine, undergo rapid degradation in whole blood, particularly at room temperature (RT). In routine casework, sample storage and transport conditions prior to arrival at the laboratory are frequently unknown and may vary considerably. Previous controlled studies have proposed endogenous biomarkers reflecting blood storage conditions. Their integration into routine FT analysis may allow estimation of blood sample integrity in individual cases.

Objectives: Adenosine 5′-monophosphate (AMP), phosphorylcholine (Chop), and glutathione (GSH) had previously been identified as potential endogenous biomarkers indicative of blood sample storage (e.g. AMP < 4'500 ng/mL indicate integrity issues). Here, quantification of AMP, Chop, and GSH within the laboratory's untargeted screening method was validated (ANSI and GTFCh guidelines), and its performance evaluated using 489 authentic FT whole-blood samples.

Methods: Accuracy/precision were assessed in water due to the endogenous character of the biomarkers and in blood (3 QC levels, triplicates, n=5 days). Of the 489 fluoride-stabilized blood samples (Lind-Vac®, 0.25% NaF), 50 were collected by physicians of the Zurich Institute of Forensic Medicine (ZIFM) and transported refrigerated, while transport conditions for all remaining samples were unknown. Blood samples were routinely analyzed using LC-HRMS (protein precipitation, reversed-phase chromatography; Impact II QTOF, ESI+, DIA), including quantification of AMP, Chop, and GSH. Retrospective anonymized data analysis was performed (ethics waiver Req-2026-00459). Concentration–time trends were evaluated by plotting biomarker concentrations against transport duration (td), defined as days between blood sampling and arrival at ZIFM. Biomarker concentrations were categorized as above/below cut-offs (provisionally established based on previous controlled studies), indicating uncooled storage > 2 days as improper based on known/observed cocaine degradation. Agreement (%) between td (< or > 2 days) and biomarker-based classification was evaluated.

Results: All biomarkers met acceptance criteria for accuracy and precision (≤ 20%) during validation. Analysis of the 489 authentic samples revealed mean time-dependent concentration trends consistent with those observed under controlled conditions. Chop showed discriminatory power only when td ≥ 10 (n=3). GSH performed best for tds ≤ 2 (82%), whereas AMP indicated superior performance for tds ≥ 7 (90%). Combined, AMP and GSH classified 91% of samples with td ≤ 2 as properly stored and 75% with td ≥ 7 as improperly stored during transport. For samples with td between 3 and 6, only 36% were classified as improperly stored; however, within the same td-range, 86% of ZIFM-samples with known proper transport were correctly classified as adequately stored.

Discussion: Td alone cannot reliably classify a sample as sufficiently intact for FT analysis and interpretation, as storage time at RT is usually unknown. Assuming uncooled transport, the combination of AMP/GSH as storage biomarkers classified samples with tds ≤ 2 or ≥ 7 as expected. For storage durations of 3–6 days, agreement between td and biomarker concentrations was low in samples with unknown storage conditions, but substantially higher in proper storage group (ZIFM), supporting the general suitability of the proposed biomarkers. This again highlights the influence of transport temperature and underscores the need for biomarkers reflecting both td and temperature to enable correct quantification and interpretation of the results.

Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

O-008

Native Peptide Analysis for the Detection of Sophisticated Synthetic Urine Products

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Abstract

Introduction: Urine samples are the matrix of choice for broad untargeted drug screenings employed in forensic/clinical toxicology. To circumvent positive drug-testing results, urine sample manipulation attempts might be carried out e.g., by means of sample dilution, sample replacement or chemical adulteration. For this purpose, the market offers a wide range of sophisticated synthetic urine products, claiming to be undistinguishable from authentic, drug-free urine samples in validity- and routine tests and often actually are (e.g., including characteristic small endogenous molecules like creatinine, uric acid and indolylacryloylglycine (IAG)).

Objectives: The aim of the current study was to differentiate a powdered synthetic urine product (TestClear urine simulation with powdered urine kit) from authentic urine samples based on a quick and simple proteomics-like workflow, where peptides natively present in the urine are analyzed without time-consuming protein digestion.

Methods: A powdered synthetic urine product (claiming to be made from real dehydrated human urine) was characterized and compared to authentic urine samples (n=10) with the laboratory's routine screening workflows (including LC-MS/MS-based creatinine quantification) in addition to UrineCheck7 dipstick tests, pH measurements and fluorometric protein quantification (Qubit). For the analysis of native peptides present in the urine, minimal sample preparation included protein precipitation and utilization of the small molecule fraction (discarding the protein pellet) for sample clean-up (STAGE-tip). After vacuum evaporation, samples were reconstituted in mobile phase starting conditions and analyzed with a previously developed microflow LC-MS/MS proteomics-method (Shimadzu Nexera coupled to Sciex zenoTOF 7600) using gradient elution on an Acclaim PepMap™RSLC column (1x150mm) with ESI+ DIA mode (60 variable window SWATH; MS1: m/z 400-900, MS2: m/z 100-2000). Datafiles were converted to mzML format and processed using Fragpipe (V24, adapted DIA_Speclib_Quant workflow). MS/MS spectra were searched against the human proteome database (UniprotID: UP000005640; reviewed sequences, including decoys/ contaminants) for identification purposes.

Results: The synthetic urine product could not be distinguished from authentic urine samples by routine analysis. Parameters like visual appearance, pH (7.48), creatinine concentration (83 mg/dL), presence of other endogenous small molecules (e.g., uric acid and IAG) and the urine adulteration dipstick test results were all within the expected ranges. Additional global protein quantification also yielded results in the expected range of normal human physiology (0.43 µg/µL). The novel native peptide analysis workflow identified human peptides from abundant urine proteins like uromodulin and fibrinogen (alpha chain) in authentic urine samples. No human peptides could be identified in the synthetic urine product; instead, snippets of the bovine casein protein were detected.

Discussion: Given the very sophisticated nature of the synthetic urine products on the market, direct visual inspection of the sample collection process is crucial (e.g. by police officers). The newly proposed native peptide analysis workflow yielded a clear discrimination between the

synthetic urine product and authentic urine samples based on the presence/absence of human peptides. As the small molecule fraction after protein precipitation is used for the current workflow, it can be easily integrated into routine forensic toxicological screening analyses, potentially even without the need for further sample preparation/analysis (if sensitivity allows); simply by utilizing the proposed data processing approach.

Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

O-009

Ultra-Fast Detection of Drug Cutting Agent in Urine Using LDTD-MS/MS in Under 11 Seconds per Sampled

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Abstract

Introduction: The adulteration of illicit drugs is a growing public health concern, with substances such as xylazine, medetomidine, and fentanyl frequently added to increase potency or profit, raising overdose risk. Naloxone, while effective for opioid overdoses, is ineffective against newer cutting agents like xylazine and medetomidine, making rapid identification essential for appropriate treatment. To address this, a novel urine analysis method using Laser Diode Thermal Desorption-Tandem Mass Spectrometry (LDTD-MS/MS) was developed to detect fentanyl, norfentanyl, xylazine, and 3-OH-medetomidine, the primary metabolite, since less than 1% of medetomidine is excreted in its parent form[1-2].

Objectives: This study developed and validated a rapid urine sample preparation method, following Bioanalytical Method Validation Guidelines[3], that uses B-One enzymatic deconjugation to extract fentanyl, norfentanyl, xylazine, and the medetomidine metabolite, 3-OH-medetomidine. Combined with LDTD-MS/MS, the method supports high-throughput, efficient drug screening.

Methods: Each 50 µL urine sample was fortified with 10 µL of internal standard solution in methanol and mixed. Then, 50 µL of B-One enzyme (Kura Biotech) and 40 µL of water were added. The mixture was agitated at 1000 rpm for 30 seconds and incubated at room temperature for 15 minutes to allow glucuronide deconjugation. Following incubation, 225 µL of extraction buffer (1:1 mixture of 1M K_2HPO_4 and saturated NaCl) was added and mixed. Salt-Assisted Liquid-Liquid Extraction (SALLE) was performed by adding 450 µL of acetonitrile, followed by agitation at 1000 rpm (30 seconds) and centrifugation at 13,000 rpm (2 minutes). A 100 µL aliquot of the supernatant was mixed with 40 µL of desorption solution, and 5 µL of the final mixture was spotted onto a LazWell96 plate, dried, and analyzed by LDTD-MS/MS.

Results: To validate the method, calibration curves were prepared in drug-free urine at 5–200 ng/mL for fentanyl and 20–800 ng/mL for norfentanyl, xylazine, and 3-OH-medetomidine (parent form <1%). Signals were normalized using analyte-to-internal standard peak area ratios (fentanyl-d_5 and norfentanyl-d_5). Validation followed Bioanalytical Method Validation guidelines[3]. Interference at the LLOQ was <20% for all analytes, and linearity exceeded 0.99 (1/x weighting). Inter-run precision was <15% CV for all calibrators and QCs (<20% CV at the LLOQ), while accuracy was within ±15% of nominal values (±20% at the LLOQ). Matrix specificity was confirmed by analyzing six blank urine matrices, all of which tested negative. Wet stability (24 h at 4°C) and dry stability (1 h at room temperature on LazWell plates) were assessed using standards and QCs; precision and accuracy remained within 15% CV and ±15% bias under both conditions. Real samples (n=10) were cross-validated using LC-MS/MS.

Discussion: The combination of enzymatic deconjugation and LDTD-MS/MS enables high-throughput screening that accounts for conjugated metabolites, significantly reducing the occurrence of false results. Furthermore, room-temperature deconjugation minimizes analyte degradation by eliminating the need for high-temperature, long-term incubations. With an analysis

time of just 11 seconds per sample, this approach provides a rapid, reliable method for clinical drug testing, enabling timely targeted interventions for life-saving treatment.

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Disclosure

Yes, I, or a member of my immediate family, has a financial interest to disclose.

Conflict of Interest

Salary/Consultant

O-010

Development of an Automated Extraction and Quantitative LC-MS/MS Method for Prescription and Over-the-Counter Medications in Blood DWI Cases

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Abstract

Introduction: Widespread availability of prescription and over-the-counter (OTC) medications is often underestimated for their role in driving while impaired (DWI) cases, death investigations, and interpretation of polysubstance interactions. Individuals frequently believe these medications are safe since they are prescribed from their doctors or readily accessible. These drugs intended for the treatment of depression, anxiety, allergies, insomnia, musculoskeletal pain, and/or seizures may contribute to toxic polysubstance effects, central nervous depression, or impairment to cognition and motor skills.

Objectives: The purpose of this study was to develop an automated extraction of blood samples for the quantitative liquid chromatography-triple quadrupole mass spectrometry (LC-MS/MS) analysis of the following analytes: amitriptyline, carisoprodol, chlorpheniramine, cyclobenzaprine, desipramine, diphenhydramine, doxylamine, gabapentin, hydroxyzine, imipramine, meprobamate, nortriptyline, pregabalin, sertraline, venlafaxine, and zolpidem.

Methods: Calibrators, controls and samples (250 µL) were pipetted into test tubes by the analyst. Automated extraction was streamlined using the Integra ASSIST PLUS pipetting robot. The program began with a 750 µL addition of a deuterated internal standard (ISTD) mixture in acetonitrile to each blood sample for protein precipitation. Tip-on-tip (ToT) cleanup filtered the supernatant via DPX Low Porosity <1 µm Filtration tips attached to the bottom of Integra 1250 µL tips for the removal of matrix interferents and particulates. Samples were further extracted by aspirating and dispensing three times on 1250 µL Integra tips containing DPX XTR 20 mg, 60 µm weak anion exchange (WAX) sorbent material. Extracts were analyzed using a Phenomenex Kinetex C18 column (2.6 µm, 50 x 4.6 mm) by Agilent 1290 Infinity II liquid chromatograph coupled with Agilent G6470B triple quadrupole mass spectrometer in positive electrospray ionization mode.

Results: The final gradient was a linear ramp for 7.5 minutes from 20% mobile phase B (0.1% formic acid in methanol) to 98% B. Mobile phase A was water with 0.1% formic acid, and the flow rate was 0.7 mL/min with a 2 µL injection volume. Two dynamic multiple reaction monitoring (dMRM) ion transitions and collision energies were identified for all target analytes and ISTDs followed by source optimization of the capillary voltage (3000V), gas temperature (200°C), gas flow (10L/min), nebulizer pressure (50psi), nozzle voltage (0V), sheath gas heat (400°C), and sheath gas flow (12L/min). Linear calibration curves with a R2 of 0.99 or greater were determined for each analyte ranging from 10/50/100/500–600/3000/6000/30000 ng/mL.

Discussion: A quantitative LC-MS/MS method was developed and optimized to quantify common prescription and OTC medications in blood. Validation and implementation of this method would expand the scope of analysis and consolidate the target analytes from two gas chromatograph-mass spectrometer (GC-MS) methods into one quantitative LC-MS/MS method.

Total run time would decrease from up to 28.5 minutes per sample on GC-MS to 11 minutes on LC-MS/MS. Automation, DPX ToT filtration and WAX solid phase extraction tips would increase sample preparation efficiency by reducing extraction time from approximately 3 hours to 1 hour in comparison to the current case workflow for analyzing these analytes by non-automated techniques.

Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

O-011

Ion Mobility-Enhanced Broad-Scope Forensic Toxicology Screening in Urine

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Abstract

Introduction: Forensic toxicology screening must continuously adapt to a rapidly changing landscape of toxicants, including new psychoactive substances (NPS). Liquid chromatography coupled to high-resolution mass spectrometry (LC-HRMS) provides high sensitivity and throughput but is commonly applied in targeted workflows, detecting predefined analytes. This leads to a need for multiple methods for broad coverage and potential false negatives when emerging compounds or metabolites are absent from target lists. Reliable detection and defensible identification in biological matrices therefore remain analytical challenges. Integrating ion mobility (IM) with mass spectrometry could enhance selectivity and confidence in identifications by enabling determination of the collision cross-section (CCS) of an analyte from its mobility behaviour. When combined with HRMS data, CCS provides an additional identification parameter that can support compound annotation and is generally more reproducible than chromatographic retention times. Accordingly, LC-IM-HRMS represents a promising but still under-implemented approach in routine forensic toxicology casework.

Objectives: A comprehensive non-target liquid chromatography–drift tube ion mobility–quadrupole time-of-flight mass spectrometry (LC-DTIM-QTOF-MS) workflow is developed and validated for general urine screening. The added value of drift time and CCS information for toxicological compound identification is evaluated.

Methods: Validation was performed using 100 µL human urine prepared by protein precipitation with 480 µL ACN and reconstitution in mobile phase and analysed on an Agilent 1290 Infinity II UPLC coupled to an Agilent 6560 DTIM-QTOF-MS. Chromatographic separation was achieved on a biphenyl column (2.6 µm, 100 x 2.1 mm) using a 28-min gradient. Analyses were performed in data-dependent acquisition (DDA) mode. An in-house library was generated including retention time, precursor accurate mass, isotopic pattern, diagnostic fragment ions, and $^{DT}CCS_{N2}$ values.

Results: The optimised LC-DTIM-QTOF-MS method enabled broad-scope screening of urine extracts following simple protein precipitation and was validated for 50 compounds. Reproducible retention was achieved across relevant toxicological classes, including cathinones, opioids, benzodiazepines, and phenethylamines, with retention time RSD < 3%. Matrix effects ranged from 69% to 111%, while acceptable selectivity was achieved with no significant carryover. Incorporation of DTIM provided reproducible drift time and $^{DT}CCS_{N2}$ values in addition to more conventional HRMS identification parameters. $^{DT}CCS_{N2}$ values showed RSDs < 1%, supporting their use as robust supplemental descriptors under controlled DTIM conditions. In selected isomeric compounds, drift time and $^{DT}CCS_{N2}$ values enabled additional discrimination. The cathinone positional isomers 2-MMC, 3-MMC, and 4-MMC showed reproducible $^{DT}CCS_{N2}$ trends (2-MMC <4-MMC< 3-MMC), with differences of 0.5–1.4%, while only partially resolved under the chromatographic conditions. Likewise, para-Fluorofentanyl and ortho-Fluorofentanyl were resolved chromatographically and by ion mobility, with $^{DT}CCS_{N2}$ differences of ≥1%.

Discussion: The developed LC-DTIM-QTOF-MS workflow demonstrated suitability for broad-scope toxicological screening in urine. Integration of DTIM increased analytical selectivity by introducing drift time and $^{DT}CCS_{N2}$ values as complementary identification parameters to accurate mass, isotope pattern, retention time, and MS/MS fragmentation. This was particularly valuable for isomers, where insufficient chromatographic separation and overlapping MS/MS spectra can limit confident differentiation. Expansion of the in-house library, including $^{DT}CCS_{N2}$ values, as well as implementation of untargeted data analysis workflow to extend screening coverage of analytes is ongoing.

Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

O-012

Ion-Exchange Online-SPE for the High-Throughput Analysis of Multi-Class Drugs in Human Plasma

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Abstract

Introduction: The need for accurate, sensitive detection of drugs in biological matrices remains relevant for all forensic and clinical toxicology laboratories. However, the influx of novel substances and increased sample loads has introduced the need for innovative technology to accommodate complex workflows. Conventional LC-MS/MS workflows are often limited by sample preparation techniques as well as instrument idle time. Multiplexed liquid chromatography (LC) systems combined with online charge-exchange solid-phase extraction (SPE) offer a broad and reliable solution by increasing efficiency, reducing matrix effects, and maintaining analytical performance.

Objectives: Here we present a summary of our evaluation of a multiplexed four-channel online SPE-LC-MS/MS system for the quantitative determination of 35 select drugs routinely encountered in forensic toxicology casework. Using human plasma samples spiked with our drugs of interest, we assessed improvements to analytical throughput, sensitivity, and extraction recovery using newly developed online-SPE column chemistries.

Methods: A Shimadzu Nexera QX four-channel LC with a multi autosampler configuration was coupled to a Shimadzu LCMS-8060NX system. Online SPE was performed using two novel prototype columns: a weak cation exchange (WCX) column for basic drugs (amines and opioids), and a phenyl-based column for hydrophobic compounds (benzodiazepines). Samples comprised of a human plasma matrix were introduced to acetonitrile for protein precipitation. Resulting supernatant was spiked with analytes and analyzed across a concentration range of 0.05 to 200 pg/µL. Chromatographic separation was achieved using a Shim-pack Scepter C18 analytical column. Detection and quantification were performed under optimized MRM conditions.

Results: The method demonstrated excellent linearity for all of the target analytes, each with R^2 values greater than 0.998. Additionally, quantitative accuracy for each compound was found to be within ±15% across all low, medium, and high levels of quality controls. In terms of throughput improvement, the multiplexed system significantly reduced the overall analysis time from 8 minutes per sample using a single-stream configuration to approximately 4.7 minutes per sample using parallel stream analysis. Furthermore, our use of both WCX and phenyl-phase online extraction media effectively simplified sample cleanup without requiring manual changes to the columns or mobile phases.

Discussion: We have demonstrated that multiplexed online SPE-LC-MS/MS provides a robust and efficient solution for high-throughput, reliable analysis of drug compounds in complex biological matrices. Using multiple SPE chemistries enabled us to effectively target a broader scope of analytes, while maintaining strong analytical performance. Increases to throughput without sacrificing sensitivity or accuracy makes this workflow highly applicable to forensic and clinical laboratories where rapid turnaround times are critical and high sample loads are routinely experienced. This approach provides a unique technical advantage compared to conventional workflows and is scalable for multi-class drug analysis.

Disclosure

Yes, I, or a member of my immediate family, has a financial interest to disclose.

Conflict of Interest

Salary, I am a paid employee of Shimadzu Scientific Instruments.

O-013

50-Year Retrospective and Appreciation: Journal of Analytical Toxicology (JAT)

Dayong Lee[1,2], Frank Peters[1,3]

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Abstract

Now in its 50th year, the Journal of Analytical Toxicology (JAT) stands as a cornerstone of forensic and clinical toxicology, tracing a trajectory of scientific rigor, innovation, and community service since its inaugural issue in 1977 under founding Editor-in-Chief Dr. Randall C. Baselt. Throughout its evolution from PubMed/MEDLINE indexing beginning in 1980, early adoption of online publishing in 1996, first tracked Impact Factor in 1997, and the 2011 acquisition by Oxford University Press, JAT has continually advanced the field's analytical standards and knowledge base and provides valuable continuing education and communication platforms for toxicologists, partnering professionals, and the public.

By 2026, JAT has transitioned to a fully online format and publishes nine issues annually, reflecting growing global demand for high-quality toxicology research. As the official journal of both the Society of Forensic Toxicologists (SOFT) and The International Association of Forensic Toxicologists (TIAFT), JAT has supported scientific exchange across continents, producing more than 4,000 peer-reviewed articles read in over 100 countries. Its most-cited works including the SWGTOX standard practices for method validation, landmark studies on drug pharmacokinetics, postmortem, drug-facilitated sexual assault, and impaired driving casework, and innovative methodologies demonstrate how JAT has shaped both laboratory practice and public health policy.

The journal's success is rooted in the dedication of its editorial leaders, from Dr. Baselt's foundational vision to Dr. Bruce A. Goldberger's decades of stewardship and now continue with our commitment to build on their legacy, supported by the exceptional work of our managing editor and publisher. JAT further owes its strength to the thousands of authors, reviewers, editorial board members, associate and guest editors, and the broader SOFT and TIAFT communities whose expertise and service have defined and advanced its scientific foundation. Their contributions support professional recognition such as the Editor's Choice Articles and the SOFT's EDIT Award, reinforcing JAT's role in spotlighting excellence in toxicology research.

Together, these milestones and contributions illustrate JAT's enduring commitment to advancing forensic and analytical toxicology. The journal's 50th anniversary special collection, now available online, honors all the invaluable contributors who have influenced its growth and impact. As JAT celebrates its 50-year anniversary, coinciding with the 2026 SOFT-TIAFT joint meeting, it remains steadfast in its mission to publish impactful, relevant, and scientifically rigorous research that informs and elevates toxicology practice worldwide.

Disclosure

Yes, I, or a member of my immediate family, has a financial interest to disclose.

Conflict of Interest

Active contract with Oxford University Press as the co-editor-in-chief of the Journal of Analytical Toxicology

O-014

Beyond Illicit Drugs: Psychotropic Medications and DUID in France: Findings From the NPS ALERT (New Psychoactive Substances Analysis, Legal Enforcement, and Road Traffic) Study

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Abstract

Introduction: The impact of psychoactive substances on driving ability is a major public health concern. Roadside drug screening programs are primarily designed to detect illicit substances, focusing on a limited number of regulated drugs of abuse (DoA) such as cannabinoids, cocaine, amphetamines, and opioids. However, the role of prescribed psychotropic medications remains underexplored despite their widespread use and known cognitive and psychomotor effects.

Objectives: This ancillary study of the French nationwide NPS ALERT project, initially designed to detect NPS undetected by routine screening (Dräger DrugCheck 3000), aimed to estimate the prevalence of psychotropic pharmaceuticals among drivers and assess implications for road safety.

Methods: The study involved six forensic laboratories from six regions of France. Nationwide, police use the Dräger DrugCheck 3000 device for oral fluid testing. After initial roadside screening for regulated DoA, public prosecutors authorized laboratories to collect used devices. A total of 2,553 devices were received and pads were analysed by liquid chromatography coupled with high-resolution mass spectrometry (LC-HRMS) and tandem mass spectrometry (LC-MS/MS) to screen for a broad range of psychoactive substances, including conventional DoA, NPS, and psychotropic medications.

Results: Among the 1,023 drivers confirmed positive for psychoactive substances, 230 tested positive for at least one psychoactive pharmaceutical with driving-impairing potential, representing 9% of all tested drivers (2,553) and 22% of confirmed positives. Ninety-four drivers out of 230 tested positive exclusively for psychotropic pharmaceuticals, entirely escaping routine screening limited to regulated drugs of abuse.

The detected compounds spanned 56 molecules across 11 pharmacological classes. The most frequently identified were methadone (n= 35), ketamine (n= 34), followed by diazepam (16), pregabalin (15), cetirizine (14), tramadol (13), paroxetine (12), buprenorphine (10), venlafaxine (10), and sertraline (9).

Discussion: The identified substances include several pharmacological classes, such as opioid substitution treatments (methadone, buprenorphine), dissociative agents (ketamine), antidepressants, benzodiazepines, gabapentinoids, and certain analgesics. Psychoactive pharmaceuticals, widely prescribed and not detected by standard roadside tests, may impair alertness, reaction time, and coordination. Methadone, found in 3.4% of positive cases, is absent from standard roadside panels. Associated with an increased accident risk (OR ~2), particularly in polydrug contexts, it represents a significant detection gap. In France, driving impairment related to prescribed pharmaceuticals is addressed through a pictogram-based classification system on drug packaging. While DUID is a criminal offence, drivers may also face legal sanctions when impairment related to prescribed medications is established, although this is seldom investigated. International studies report similar trends, with increasing detection of prescription drugs often combined with other substances among drivers. These findings highlight a gap between current screening capabilities and real-world substance use patterns.

In conclusion, a substantial proportion of the population uses psychoactive pharmaceuticals that may impair driving ability and increase accident risk. This issue remains underrecognized and warrants greater attention in forensic toxicology and traffic safety policies, as well as improvements in roadside testing strategies.

Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

O-015

Comparison of Blood Alcohol Concentrations in Seriously Injured Drivers in Motor Vehicle Crashes With DUI Casework

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Abstract

Introduction: Driving under the influence (DUI) of alcohol remains a leading cause of motor vehicle crash (MVC) fatalities and serious injuries, leading to a focus of enforcement, prosecution, and prevention by criminal justice and public health systems. A significant decrease in DUI arrests by police agencies in Miami-Dade County, Florida, from 2009 to 2016, compared to the average across Florida and the United States, was reported. This study evaluated the prevalence and concentration of alcohol among deidentified MVC patients admitted to Ryder Trauma Center's Trauma Resuscitation Unit (Ryder TRU) in Miami-Dade County for treatment and compared blood alcohol concentrations (BAC) to DUI investigations in Miami-Dade County. Additionally, data were examined to determine whether there were significant differences in BAC by sex and age ranges among injured drivers and subjects in DUI investigations.

Objectives: The objective of this study is to characterize alcohol use among seriously injured drivers in Miami-Dade County and determine whether statistically significant differences in BAC exist compared to DUI casework.

Methods: MVC patients who arrived at Ryder TRU between 9/1/2025 and 3/1/2026 were assessed for inclusion. For patients included in the study, a blood sample was provided to the University of Miami Toxicology Laboratory (UMTL) for testing. Forensic blood specimens submitted to the UMTL from DUI investigations in Miami-Dade County with incident dates in the patient specimen collection period were reviewed. This study and the publication of forensic results were approved by the University of Miami IRBs under protocols 20240973 and 20040392, respectively.

Trauma study specimens were analyzed for ethanol utilizing a gas chromatograph-flame ionization detector (HS-GC-FID) with dual analytical columns and FIDs. DUI blood specimens were analyzed for ethanol using either a headspace-gas chromatograph-flame ionization detector/mass spectrometer (HS-GC-FID/MS) or by HS-GC-FID. Both methodologies had a calibration range of 0.020-0.400 g/dL and a limit of detection of 0.010 g/dL. The HS-GC-FID methodology used for testing DUI specimens and trauma samples was identical. Drug testing was also performed, but results are not discussed in this presentation.

Results: Ethanol was detected in 67/281 (23.8%) trauma patient drivers, with 55/281 (19.6%) having a BAC >0.08 g/dL. BAC in trauma patients ranged from 0.023 to 0.388 g/dL (mean: 0.165 ± 0.073 g/dL). Among DUI investigations, 31/40 (77.5%) subjects had ethanol detected, all with a BAC >0.08 g/dL, ranging from 0.125-0.358 g/dL (mean: 0.206 ± 0.062 g/dL).

A 3-way ANOVA demonstrated a statistically significant main effect of driver type ($p < 0.05$), while the main effects of sex and age were nonsignificant. Significant interactions were observed between type and sex, and between type and age. The interactions of sex by age and type by age by sex were nonsignificant.

Discussion: Alcohol was detected in nearly 25% of seriously injured MVC drivers, with most cases exceeding the legal limit, suggesting alcohol impairment as a contributor to severe MVC's. Although DUI investigation subjects exhibited significantly higher mean BAC's, trauma patients also demonstrated concentrations consistent with substantial impairment. This difference may reflect differences in enforcement rather than reduced prevalence or risk. The absence of significant sex or age main effects indicates that alcohol-related injury spans demographic groups.

Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

O-016

An Evaluation of Low Benzoylecgonine Concentrations in Urine

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Abstract

Introduction: In federally-regulated workplace urine drug testing, benzoylecgonine is analyzed to detect cocaine use. Cocaine has been detected on surfaces of paper currency and school desks, indicating the possibility of passive exposure. Indeed, benzoylecgonine concentrations up to 11 ng/mL have been observed in end-of-shift urine samples from individuals working in forensic laboratories.

Objectives: The aim was to determine the prevalence of benzoylecgonine in urine at concentrations below the U.S. Department of Health and Human Services (HHS) initial test cutoff of 150 ng/mL.

Methods: Deidentified negative urine samples (n=2,809) from federally-regulated drug testing were analyzed using liquid chromatography quadrupole Time-of-Flight mass spectrometry (LC-QTOF-MS). Samples were extracted using mixed mode cation exchange (MCX) solid phase extraction (SPE) followed by separation on a biphenyl column (Restek Raptor 100x2.1 mm, 1.8 µm). Data was collected in MS^E mode with a Waters Xevo G2-XS. The panel contained 75 analytes, including common drugs of abuse, emerging drug trends, and prevalent novel psychoactive substances (NPS). Samples were deemed positive for benzoylecgonine if they met identification criteria (retention time, accurate mass, fragments present). The estimated limit of detection (LOD) was 5,000 area counts, corresponding to ~1 ng/mL, depending on the run (range 0.2-4.9 ng/mL). Semi-quantitative benzoylecgonine concentrations were estimated using area ratios with reference samples at 5, 10, and 50 ng/mL, included in each run.

Results: In 7.4 million federally-regulated workplace urine specimens tested at HHS-certified laboratories, the positivity, compared to federal cutoffs, for benzoylecgonine was 0.15% in 2025 (≥150 ng/mL), and the total drug positivity was 2.26%. By comparison, 3.6% (n=100) of the specimens in this study were positive for benzoylecgonine, using the criteria described above. While quantifications cannot be made, most estimated concentrations were well below 150 ng/mL. The median estimated concentration was 4.1 ng/mL with a majority of results (74/100) in the 1-10 ng/mL range.

Discussion: While it is expected that some benzoylecgonine concentrations in urine from people who use cocaine will be below the 150 ng/mL initial test cutoff, but the positivity in the study is striking. These results were obtained using significantly more sensitive methods than routinely used in workplace drug testing. As such methods become increasingly prevalent in post-mortem and driving under the influence laboratories, forensic toxicologists should consider multiple possibilities for low benzoylecgonine concentrations in urine.

Most estimated benzoylecgonine concentrations were less than 10 ng/mL (79/100, including 5 specimens <1 ng/mL), which corresponds with urine levels from individuals handling cocaine

in forensic laboratories. This indicates that some benzoylecgonine positives might be from environmental exposure. However, low benzoylecgonine concentrations are also expected after cocaine use as the drug is metabolized and eliminated. The take home-message is that low urine benzoylecgonine concentrations in urine may not be proof of prior cocaine use in all cases.

Limitations of this study include that some specimens likely come from people that have used cocaine, the semi-quantitative approach, and that specimens were limited to a few laboratories and timepoints, which might not represent all federally-regulated specimens.

Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

O-017

Prevalence of Ketamine in DUI cases in Colorado 2025

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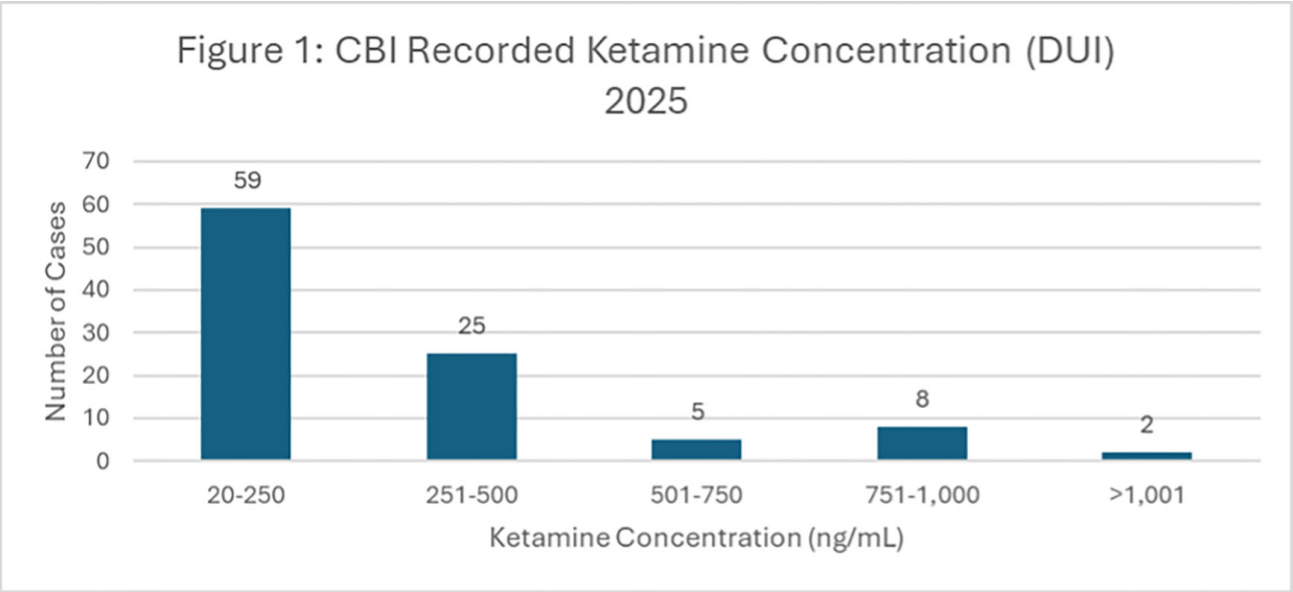
Abstract

Introduction: Ketamine is a Schedule III controlled substance commonly used in medical settings as an dissociative anesthetic. Ketamine therapy is legal in Colorado when prescribed and administered by licensed healthcare professionals for treatment of depression, anxiety, or chronic pain. While there are legitimate medical uses for ketamine, it can also be used recreationally to produce dissociative effects and hallucinations. Some general effects of ketamine are drowsiness, sedation, altered perception, dissociation, and slowed reaction time. Ketamine can be injected, consumed, snorted or added into other substances for smoking. Due to suspected increase in prevalence, in October 2024, the Colorado Bureau of Investigation (CBI) began screening all submitted Driving Under the Influence (DUI) cases for ketamine via Enzyme Linked Immunosorbent Assay (ELISA).

Objectives: To determine the prevalence of Ketamine among suspected impaired drivers in Colorado in 2025. This presentation will also examine common polydrug trends with Ketamine in Colorado.

Methods: Whole blood samples submitted to CBI for analysis in suspected DUI cases undergo ethanol/volatiles analysis and a 15-panel drug screen via ELISA, which has a ketamine cutoff of 10 ng/mL. Ketamine presumptive positive cases are confirmed via NMS Labs using Liquid Chromatography/Tandem Mass Spectrometry (LC-MS/MS) with a lower limit of quantitation of 20 ng/mL. All other presumptive positive drugs are confirmed in-house using LC-MS/MS and Gas Chromatography Mass Spectrometry (GC-MS).

Results: In 2025, CBI completed 10,048 cases, of which 99 (0.98%) were confirmed containing ketamine greater than 20 ng/mL. The average ketamine concentration was 288 ng/mL (23 ng/mL – 1,700 ng/mL). The distribution of cases across the concentration range is illustrated in Figure 1. In 59 cases the reported ketamine concentration was less than 250 ng/mL. The majority of ketamine cases were polydrug cases (92). Of the seven cases that were ketamine only, the average concentration was 472 ng/mL (74 ng/mL – 1,000 ng/mL). When evaluating ketamine only cases, six of the seven ketamine cases were not related to a crash, perhaps indicating ketamine was not medically administered. When evaluating polydrug use, ethanol was detected with ketamine in 49 cases, and 10 cases were ketamine and ethanol only. Further analysis of cases in which ketamine was reported shows cocaine (and/or metabolites) was reported in 53 cases, and cannabis (THC and/or metabolites) was detected in 45 cases.



Discussion: Ketamine was reported in less than 1% of the total cases received in Colorado in 2025. However, the data provides significant information regarding poly substance use and ketamine concentrations in DUI cases. Ethanol, cocaine and cannabis were the most used substances in conjunction with ketamine. Majority of cases were found to be at concentrations lower than 250 ng/mL, potentially due to poly drug use or rapid elimination of the substance. It was found that ketamine only cases typically contained higher concentrations of ketamine than that of poly substance cases. The prevalence of ketamine poly substance use highlights the complexity of interpreting ketamine impairment in DUI casework. Evaluating the ketamine only cases in Colorado can provide significant information regarding the effects of ketamine on impairment in DUI investigations.

Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

O-018

Mitragynine Minefield – A Retrospective Analysis of Colorado Casework With Impaired Driving Case Studies

Stephanie Olofson

Colorado Bureau of Investigation, Arvada, CO, USA

Abstract

Introduction: Mitragynine, informally known as Kratom, is the primary psychoactive component in the Southeast Asian plant *Mitragyna speciosa*. Currently, mitragynine is recreationally available with no medicinal use recognized by the Food and Drug Administration. It has been reported that mitragynine may be used for pain relief and as a treatment for anxiety and depression. It is also publicized as a treatment for opioid dependence/withdrawal as it is a partial agonist at the μ-opioid receptor. The effects of mitragynine are dose dependent. Reportedly, at low doses mitragynine has stimulant effects, while at higher doses it has depressant effects.

Colorado has limited regulations on the sale of mitragynine to include: no sales to those under age 21, guidelines for labeling and formulation, and a 2% cap on 7-hydroxymitragynine. Given conflicting federal and state regulations, limited medicinal studies, and recreational availability, it can be difficult to interpret the significance of mitragynine in DUI cases. Additional challenges are also faced due to the range of effects that may be noted plus the high rate of polydrug cases.

Objectives: This presentation will evaluate nine years of impaired driving, mitragynine positive casework from the Colorado Bureau of Investigation (CBI). The goal is to assess trends and to evaluate the effects seen in casework.

Methods: Currently, CBI only detects mitragynine in cases where it is listed as a suspected drug on submission information or if the case screens positive for another drug within our pain management confirmation. While there have been shifts in protocol, CBI currently uses Enzyme-Linked Immunosorbent Assay as the screening method to screen for buprenorphine, fentanyl, methadone, opiates, oxycodone/oxymorphone, and tramadol which are then confirmed via the pain management assay. Mitragynine confirmation is performed using solid phase extraction with instrumental analysis via liquid chromatography tandem mass spectrometry. Mitragynine is reported qualitatively using a one-point curve to 10.0 ng/mL with a 1.0 ng/mL control/decision point. Duplicate analysis is required for a case to be reported. Cases can be reported below 1.0 ng/mL if acceptance criteria are met.

Results: Between 2017-2025, CBI had 232 mitragynine positive cases, 230 were suspected impaired driving cases. Demographically, the ages range from 16-67 years old and 75% of the drivers are male. In regards to detection, 21% of cases were assigned a mitragynine screen based upon submission information and the remainder were detected via the pain management assay. Most recognizably, 97% of mitragynine cases are polydrug cases. Other co-reported drugs, in order of prevalence, are other opioids, benzodiazepines, ethanol, cannabinoids, and sympathomimetic amines.

While CBI reports mitragynine qualitatively, there is value in evaluating in the qualitative levels determined in our analysis. For this study, the average of the two qualitative analyses required for confirmation ranged from 0.5 – 2,114 ng/mL. 25% of cases had a result under 5 ng/mL and the average mitragynine result for all cases was 94 ng/mL.

Discussion: This presentation will explore the trends in CBI's mitragynine positive DUI case work over a nine-year span. Highlighted impaired driving case studies, both single and polydrug, will demonstrate interpretation challenges, especially when multiple drug classes are present.

Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

O-019

Comprehensive LC-Orbitrap-MS Screening and Interpretation of Polydrug Exposure in Post-Mortem DUID Cases

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Abstract

Introduction: The increasing diversity of therapeutic drugs, illicit substances, and new psychoactive substances (NPS) represents a major analytical challenge in forensic toxicology. High-resolution mass spectrometry (HRMS) enables broad-spectrum screening; however, the interpretation of complex polydrug exposure remains a critical limitation, particularly in post-mortem DUID cases.

Objectives: To apply a comprehensive LC-Orbitrap-MS workflow for detection and toxicological interpretation of polydrug exposure in post-mortem DUID cases.

Methods: A total of 128 post-mortem whole blood samples from DUID cases were analyzed using a LC-Orbitrap-MS screening method. Whole blood samples (300 µL) were fortified with internal standard (MDMA-d_5) and diluted with saturated sodium tetraborate solution. Liquid-liquid extraction was performed using methyl-tert-butyl ether:isopropanol:hexane (5:3:2, v/v), followed by agitation cycles, centrifugation, freezing-assisted phase separation (–80 °C), evaporation under nitrogen, and reconstitution in mobile phase prior to analysis. Data acquisition included full-scan HRMS and data-dependent MS/MS fragmentation. Compound identification was based on retention time, isotopic pattern, spectral library matching (ToxExplorer™ database), and fragmentation profile. Data were further processed using a structured analytical pipeline including compound classification, toxicological relevance attribution, co-occurrence analysis, and multivariate statistical approaches. This study was approved by the Institutional Ethics Committee of the University of Campinas (UNICAMP) (CAAE: 84492624.3.0000.5404).

Results: A wide range of substances was detected, including illicit drugs, NPS, pharmaceuticals, metabolites, and adulterants. High toxicological relevance compounds were frequently detected, particularly midazolam (n=26), cocaine (n=25), fentanyl (n=23), benzoylecgonine (n=19), cocaethylene (n=16), and etomidate (n=15). Several fentanyl analogs and dissociative agents such as deschloroketamine and ketamine were also commonly observed. Adulterants were highly prevalent, especially benzocaine (n=103) and lidocaine (n=45), indicating extensive drug adulteration. Polydrug exposure was a prominent feature, with up to 16 high-relevance compounds and 21 toxicologically relevant substances detected in a single case, frequently involving multiple pharmacological classes. The most frequent critical combinations included cocaine with local anesthetic adulterants (n=24), opioids with benzodiazepines or sedatives (n=21), synthetic opioids

with illicit drugs or NPS (n=20), cocaine with cocaethylene (n=16), and dissociatives with CNS depressants (n=10). Marker-based analysis revealed high prevalence of MDMA-like compounds (n=124), adulterants (n=113), benzodiazepines (n=33), cocaine (n=25), fentanyl or analogs (n=23), and dissociatives (n=17), highlighting overlapping patterns of stimulant, opioid, and sedative use.

Discussion: The results demonstrate a high complexity of toxicological findings in post-mortem DUID cases, characterized by extensive polydrug exposure and frequent detection of high-risk drug combinations associated with impaired driving and increased fatality risk. The integration of LC-Orbitrap-MS with database-assisted identification enabled comprehensive detection of both targeted and non-targeted compounds, including emerging NPS and adulterants. The high prevalence of metabolic markers such as benzoylecgonine and cocaethylene reinforces the importance of HRMS for confirming drug intake and co-exposure patterns. This approach provides a robust framework for large-scale toxicological screening and supports improved interpretation of complex forensic cases, contributing to toxicovigilance and public health strategies.

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Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

O-020

Evaluating Two Years of Impaired Driving Surveillance in Arkansas through the Arkansas State Crime Laboratory–Glen F. Baker Public Health Laboratory Collaboration

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Abstract

Introduction: Many forensic laboratories face significant limitations in personnel, budget, and analytical capacity, which restrict their ability to perform comprehensive drug testing on every impaired driving case. As a result, many laboratories employ stop-limit testing, in which drug analysis is not conducted when a driver's blood alcohol concentration (BAC) exceeds a designated threshold, typically at or above the per se limit. Although efficient, this approach leaves gaps in understanding the true prevalence of drug involvement among impaired drivers. The National Safety Council (NSC) recommends that forensic toxicology laboratories test for impairing drugs and their metabolites in suspected impaired driving cases. Without such data, assessing the extent of drugged driving and evaluating the crash risk associated with individual substances or combinations of substances is challenging.

To address the lack of statewide drug use data among impaired drivers in Arkansas, the Arkansas State Crime Laboratory (ASCL) and the Glen F. Baker Public Health Laboratory (GFBPHL) launched the Impaired Driving Surveillance Project in June 2024. Through this collaboration, the GFBPHL provides rapid toxicology testing and reporting, supplying ASCL, law enforcement, policymakers, and healthcare partners with more immediate and actionable information in a rapidly shifting drug landscape.

Objectives: The primary objective of the Impaired Driving Surveillance Project is to better characterize the prevalence of polysubstance impairment among Arkansas drivers. Additionally, the project aims to serve as a model for how state laboratories can collaborate to expand capabilities, enhance surveillance, and strengthen public health and safety initiatives.

Methods: The ASCL provides the GFBPHL with de-identified blood samples from impaired driving casework that fall under the ASCL's stop-testing procedures. After initial testing at the ASCL, a sub-aliquot of each sample is collected, de-identified, and transferred to the GFBPHL for additional analysis. Samples are prepared using supported liquid extraction (SLE) and analyzed on a Thermo Scientific Orbitrap Exploris 480, generating full-scan high-resolution MS and unit-resolution MS2 data. Multi-drug and cannabinoid screening methods use targeted compounds recommended in the NSC's Tier I and Tier II panels. Reference materials analyzed previously were used to build an in-house MS2 spectral library to support compound identification. All results are reported qualitatively.

Results: In 2024, 128 samples were tested, with 61 positives for at least one drug; approximately 50 percent involved polysubstance use. CNS depressants (32 percent) and CNS stimulants (19 percent) were the most frequently identified classes. In 2025, 358 samples were submitted, with 241 testing positive, resulting in a 67 percent positivity rate. Cannabinoids, potentially impairing neuropsychiatric medications (PINM), and CNS depressants were the most common categories detected. More than one drug class was present in 105 samples, and two samples contained five or more classes. THC and its metabolite were the most frequently detected Tier 1 compounds.

Discussion: Overall, the 2024–2025 results demonstrate a clear and rising concern regarding polysubstance impairment among Arkansas drivers. While 2026 data remain incomplete, early indications suggest that these trends will continue. These findings highlight the importance of sustained, coordinated efforts among public health partners, forensic laboratories, policymakers, and law enforcement to reduce impaired driving and improve roadway safety across the state.

Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

O-021

Prevalence of Psychoactive Substances Among French Drivers: Key Findings From the NPS ALERT (New Psychoactive Substances Analysis, Legal Enforcement, and Road Traffic) Study

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Abstract

Objectives: In France, while 12% of drivers involved in fatal accidents test positive for regulated drugs of abuse (DoA) (cannabis, cocaine, amphetamines, and opioids), current roadside screening remains limited to these four categories of substances, leaving emerging substances such as new psychoactive substances (NPS) undetected despite their increasing prevalence. The NPS ALERT study was therefore designed to estimate the prevalence of NPS and other psychoactive substances among French drivers, and to analyze the effectiveness of current screening strategies, with a view to informing road safety policy.

Methods: Following approval by the public prosecutor, a total of 2,553 oral fluid (OF) specimens were collected by law enforcement across six French regions (Auvergne-Rhône-Alpes, Hauts-de-France, Grand-Est, Île-de-France, Nouvelle-Aquitaine, and Provence-Alpes-Côte d'Azur). Used roadside screening devices (Dräger DrugCheck 3000) were collected continuously throughout 2025 by law enforcement officers and subsequently transferred to 6 forensic laboratories for confirmation analyses using liquid chromatography coupled with tandem mass spectrometry and liquid chromatography coupled with high-resolution mass spectrometry. Specimens were analyzed for conventional DoA, NPS, and Ketamine. Data processing and statistical analyses were performed using Claude Code (Anthropic, Opus 4.6 model), an agentic command-line tool integrated within Visual Studio Code under continuous human supervision.

Results: Laboratory confirmation identified 1,023 positive specimens for psychoactive substance (pos SPA group) out of 2,553 (40.1%), compared to only 20.6% positive by roadside screening using the Dräger DrugTest 3000 device. A total of 918 regulated DoA were identified (36% of the total group and 90 % of the pos SPA group), 230 psychotropic drugs (9% total group; 22% pos SPA group) and 7 NPS (0,3% total group ; 0.7% pos SPA group). A single driver may test positive for multiple categories. Among regulated substances, cannabinoids were the most frequently detected class (26.6% of all tests), followed by cocaine (13.4%), amphetamines (2.8%), and opioids (1.6%). Regional variations were observed, with cocaine prevalence peaking in Marseille (18.7%) and amphetamines showing higher rates in Grenoble (5.0%) and Lille (4.1%). Seven drivers tested positive for NPS (0.3%), primarily synthetic cathinones (2, 3 or 4-MMC) and dissociative substances (2F-DCK, O-PCE). Critically, two of these drivers had a negative roadside screening result, including one individual who had consumed exclusively dissociative NPS with no co-

detected regulated substance, thereby completely evading detection. Five of the seven drivers also had at least one regulated drug confirmed by laboratory analysis. Ketamine, not detected by current roadside devices, was confirmed in 34 drivers (1.33%). While some cases may reflect therapeutic esketamine exposure, prescription remains uncommon in France and requires medical supervision and next-day driving abstinence is explicitly advised. Furthemore, the presence of concomitant drugs of abuse in 25 drivers strongly suggests recreational use. Critically, 16 of 34 (47%) tested negative on roadside immunoassay and 7 (20%) showed no associated substance, revealing a substantial gap in the current drug-impaired driving detection framework.

Discussion: While NPS were detected at a low prevalence (0.3%), ketamine emerged as a major blind spot in roadside screening, with 1.3% of drivers testing positive corresponding to approximately 1 in 75 drivers. These findings suggest that expanding screening to include NPS is not currently justified given their low prevalence, whereas targeting ketamine appears relevant.

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No, I, nor any member of my immediate family, has a financial interest to disclose.

O-022

Old is Gold: Texas Breath Alcohol Testing

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Abstract

Introduction: Breath alcohol testing is a fast, non-invasive method, widely used in the United States to measure breath alcohol concentration (BrAC). It is primarily used by law enforcement to counter impaired driving as well as for workplace compliance. Law enforcement agencies across various states and regions along with NHTSA have established procedures and legal limits to ensure consistent application across the country. Typically, a BrAC or BAC of 0.08 g/210L or higher is considered impaired for drivers aged 21 and over, with stricter limits for commercial drivers and individuals under 21.

Breath testing devices, such as portable breathalyzers and evidential instruments estimate BrAC by analyzing the alcohol present in a person's breath providing a quick and non-invasive alternative method for testing alcohol concentration. Recent advancements including the Driver Alcohol detection system for Safety (DADSS) also improves alcohol detection through passive in-vehicle technologies.

In Texas, breath alcohol testing in driving while intoxicated cases are governed by implied consent law and administrative rules that specify procedural safeguards, including certified operators, technical supervisors, and instrumentation. These measures are intended to improve the accuracy, consistency and legal defensibility of breath alcohol results. Enforcement is carried out by TX DPS, county agencies and local police departments which follows both federal and state guidelines. The state currently utilizes the Intoxilyzer 9000 (CMI Inc) to perform breath alcohol testing.

Objectives: To review the history of the breath alcohol program and discuss specifics of the TX BrAC program and the role SWIFS plays.

Methods: Breath cases analyzed by the TX BrAC program were reviewed to provide summary case statistics and trend analysis.

Results: The Texas Breath Alcohol Program operates through 388 approved testing locations with 8030 certified operators representing 1724 law enforcement agencies with 33 Technical supervisor (TS) areas divided across the state. Southwestern Institute of Forensic Sciences (SWIFS) was involved with Breath testing as a local program since mid-1970. SWIFS is assigned as TS area 023 including major counties across the DFW metroplex. 718 subject tests were performed by SWIFS TS area 023 for the year 2025, while the state as a whole performed approximately 31,600 tests. Cases will be discussed.

Discussion: Breath and breath testing are two primary methods used by law enforcement to determine intoxication. Both are designed to measure alcohol concentration in the body at or near the time of incident. Blood testing is generally considered the more comprehensive option, as it not only measures alcohol but can also detect the presence of other impairing drugs. However, breath testing offers a significant advantage in that it provides immediate results, allowing officers to make real-time decisions during traffic stops. Even though breath testing is not as popular as blood testing, it remains a reliable, practical, and cost-effective tool that plays a vital role in enforcement, public safety and legal processes related to impaired driving.

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No, I, nor any member of my immediate family, has a financial interest to disclose.

O-023

Evidence Based Policy: The Role of Oral Fluid Testing in Drug Impaired Driving Legislation in New Zealand

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Abstract

Introduction: On-site/roadside drug testing has become an essential requirement for law enforcement to assess recent drug use and driver impairment (Verstraete 2005). Conventional biological matrices such as blood and urine present practical and forensic limitations, necessitating the exploration of alternative specimens (Kintz and Samyn 2002). Oral fluid has emerged as a reliable matrix due to its resistance to adulteration, non-invasive and straightforward collection, and suitability for rapid, on-site presumptive analysis within legal frameworks (Drummer 2006). In New Zealand, the development of drug-driving legislation has spanned over two decades, driven by the need to address emerging patterns of drug impaired driving alongside alcohol related risks.

Objectives: This study aims to: (i) present the historical evolution and implementation of oral fluid testing (OFT) legislation in New Zealand; (ii) evaluate its role in enhancing road safety by targeting drug-impaired driving; and (iii) evaluate key challenges and prevalent misconceptions regarding OFT among the public and legal stakeholders.

Methods: Recent amendments to the Land Transport Act, implemented in December 2025, introduced OFT-based roadside drug testing in New Zealand. Epidemiological data indicate that drug impaired driving contributes to a substantial proportion of road fatalities, comparable to alcohol related incidents. Roadside OFT employs saliva as an alternative matrix for detecting recent drug use, with screening devices capable of presumptively identifying substances such as THC, MDMA, methamphetamine, and cocaine. Information regarding public and stakeholder perceptions of the OFT program was collected during roadside operations from comments made by drivers who refused oral fluid testing.These anecdotal observations helped identify common concerns, misconceptions, and acceptance issues. Oral fluid samples collected during the program were submitted for laboratory analysis.

Results and Discussion: The need for drug impairment testing in drivers has been recognized by forensic toxicologists in New Zealand since the late 1990s. Legislative developments began in 2009, supported by data (2003–2008) showing that approximately 51% of deceased drivers in fatal crashes had consumed impairing drugs. Subsequent modifications to alcohol limits (2011–2015) and analysis of crash data (2013–2018) further underscored the necessity for drug testing frameworks. The implementation of OFT legislation in December 2025 represents a significant advancement in road safety strategy. Author's laboratory now analyses oral fluid samples for up to 25 controlled substances (called listed drugs), with defined threshold concentrations for infringement purposes. A validated LC–MS/MS method (ANSI/ASB Standard 036, 2019) enables simultaneous detection and quantification of these analytes in a single analytical run. Supported by the available scientific literature, stakeholders were informed that the proposed cut-off concentrations were appropriate for the objectives of New Zealand's roadside OFT program. Concerns regarding the potential impact of passive drug exposure and chronic drug use on

roadside test results were reviewed and addressed through evidence presented in the expert panel report. In addition, public concerns regarding the possible misuse of collected oral fluid samples, including the use of residual DNA for purposes other than drug testing, were clarified and addressed to improve transparency and public confidence. This work highlights the scientific, legal, and public safety rationale underpinning OFT implementation and discusses its future goals in mitigating drug impaired driving in New Zealand.

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No, I, nor any member of my immediate family, has a financial interest to disclose

O-024

Determinants of Tetrahydrocannabinol and Cocaine in Oral Fluid

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Abstract

Introduction: Oral fluid is increasingly used in forensic and roadside toxicology because collection is non-invasive, directly observed, and simpler than blood sampling. However, drug concentrations in oral fluid depend strongly on pre-analytical factors, including the collection device, the extracted fraction, and specimen composition. This may be especially relevant for delta-9-tetrahydrocannabinol (THC), a highly lipophilic analyte, whereas cocaine-related compounds may be influenced by different mechanisms.

Objectives: The aim of this study was to investigate the impact of buccal-cell content and pre-analytical fractionation on measured concentrations of THC, cocaine, benzoylecgonine (BZE), and ecgonine methyl ester (EME) in oral fluid collected during routine roadside drug testing.

Methods: Routine oral-fluid specimens collected with swabs (Floqswab®, Copan) were integrated into the laboratory workflow. After immersion in phosphate buffer, sonication, and vortex mixing, a homogenate was obtained. For THC, the homogenate was centrifuged to generate a supernatant and a cell-rich pellet. For cocaine-related analytes, the swab was centrifuged separately to obtain an expressed swab fraction. THC was quantified using an online SPE LC-MS/MS method with a limit of detection (LOD) of 0.15 ng/mL. Cocaine, BZE, and EME were measured using a validated LC-MS/MS method with an LOD of 1.75 ng/mL. Buccal epithelial cells were counted in paired homogenate and pellet fractions using a Malassez-type counting chamber.

Results: A total of 102 paired specimens were available for the THC/cellularity analysis. Median buccal epithelial cell counts increased from 412,500 cells/mL in the homogenate to 1,950,000 cells/mL in the pellet, while median THC concentrations increased from 3.48 to 14.27 ng/mL. The median pellet-to-homogenate ratio was 4.40 for cell counts and 3.93 for THC concentrations; the corresponding mean ratios were 4.51 and 4.22 (both differences with $p < 0.001$). The association between cellular enrichment and THC enrichment was moderate overall (Pearson $r = 0.48$), but became strong for homogenate THC concentrations between 5 and 15 ng/mL ($r = 0.77$) and very strong above 15 ng/mL ($r = 0.88$). Four specimens were below the THC LOD in the homogenate but above it in the pellet. For cocaine-related analytes, concentrations in the homogenate, supernatant, and pellet were broadly comparable, whereas the expressed swab fraction consistently yielded the highest concentrations. Median expressed swab/homogenate ratios were 1.16 for cocaine, 1.28 for BZE, and 1.14 for EME. The expressed swab fraction reclassified 5 cocaine, 2 BZE, and 1 EME specimens from below to above the analytical threshold.

Discussion: For THC, buccal epithelial cells appear to act as a reservoir and increase concentrations in the pellet. For cocaine-related analytes, the main issue appears to be incomplete release from the swab rather than cellular enrichment. These results have practical consequences for forensic interpretation, because oral-fluid concentrations may vary not only with drug exposure, but also with specimen cellularity or swab-related retention, thereby affecting low-level positivity and discordant findings. These findings challenge the credibility of fixed oral-fluid concentration cut-offs, as measured values partly depend on pre-analytical factors independent of true systemic exposure, underscoring the need for tailored handling to improve sensitivity, reliability, and forensic interpretability in DUID testing.

Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

O-025

Preparation of A-Series Nerve Agent – Tyrosine Adduct Standards for Forensic LC-MS/MS Analysis

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Abstract

Introduction: A-series nerve agents, also known as Novichok agents, are important targets in forensic toxicology and chemical terrorism investigations. A230, A232, and A234 are representative compounds within this agent class. Because nerve agents rapidly form covalent adducts with biomolecules, protein-adduct analysis is useful for retrospective exposure verification. Although serine-adduct biomarkers are well established, albumin-derived tyrosine adducts provide complementary evidence. However, analytical studies of A-series tyrosine adducts have been limited, partly because suitable reference standards are difficult to obtain.

Objectives: The objective of this study was to develop a practical synthetic method for representative A-series tyrosine adducts and to prepare reference standards applicable to liquid chromatography-tandem mass spectrometry (LC-MS/MS) analysis. The target compounds were A232-, A234-, and A230-tyrosine adducts.

Methods: A synthetic route to A232-, A234-, and A230-tyrosine adduct standards was developed from protected tyrosine derivatives and A-series-related phosphorus intermediates. Dichloridate precursors were reacted with the corresponding amidine and then coupled with N-Cbz-L-tyrosine benzyl ester to form protected adduct intermediates. Solvent and base effects in the coupling sequence were examined, and catalytic hydrogenolysis conditions were optimized to remove benzyl and Cbz protecting groups while minimizing side-product formation. The prepared standards were characterized by reversed-phase LC-MS/MS coupled to high-resolution mass spectrometry. For comparison with biologically generated adducts, blank serum samples spiked with A-series surrogate compounds were digested with pronase and analyzed under the same LC-MS/MS conditions. Retention times and product ion spectra were compared between the synthesized standards and the digestion-derived peaks.

Results: Reference standards corresponding to A232-, A234-, and A230-tyrosine adducts were successfully prepared. Optimization of the synthetic route showed that both the protected-tyrosine coupling step and the deprotection step were critical for obtaining the desired adducts. In the deprotection study, conventional ethanol conditions caused formation of side products, whereas modified hydrogenolysis conditions using isopropanol and pyridine suppressed the ethylated side products and improved the formation of the target adducts, especially for A234- and A230-tyrosine adducts. LC-MS/MS analysis of the prepared standards provided characteristic chromatographic peaks and product ions, including m/z 372 → 299.0791 for A232-tyrosine, m/z 386 → 244.0733 for A234-tyrosine, and m/z 356 → 214.0628 for A230-tyrosine. These standards showed retention-time and fragmentation agreement with peaks obtained from pronase-digested surrogate-spiked serum.

Discussion: This study establishes synthetic access to multiple A-series tyrosine adduct standards that had not been readily available for forensic toxicological analysis. The main significance is not only the LC-MS/MS detection itself, but also the preparation of structurally relevant reference materials that allow more confident peak assignment in biological specimens. Such standards are essential for distinguishing true exposure biomarkers from nonspecific serum-derived or digestion-derived signals. The method also supports safer method development, because surrogate-spiked serum can be used to generate comparable tyrosine-adduct biomarkers without routine handling of highly restricted authentic A-series nerve agents. Overall, preparation of A-series tyrosine adduct standards provides a practical foundation for improving retrospective verification of A-series nerve agent exposure.

Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

Conflict of Interest

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O-026

Generating an Internal Standard In Situ: A Practical Isotope-Coded Derivatization Strategy for Quantitative LC–MS/MS Analysis of Urinary THC-COOH

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Abstract

Introduction: Quantitative determination of 11-nor-9-carboxy-Δ^9-tetrahydrocannabinol (THC-COOH) in urine is widely used to verify cannabis consumption in forensic toxicology. LC–MS/MS methods typically rely on stable isotope-labeled THC-COOH internal standards to compensate for matrix effects and analytical variability. However, isotope-labeled cannabinoid standards may be expensive, tightly regulated, or difficult to obtain in some jurisdictions, including Japan. Following recent legislative amendments in Japan that newly criminalize cannabis use, reliable quantitative analysis of urinary THC-COOH has become increasingly important in forensic investigations.

Objectives: The primary aim of this study was to develop an LC–MS/MS method for quantitative analysis of urinary THC-COOH using an isotope-coded derivatization (ICD) strategy that generates an internal standard without reliance on commercially available isotope-labeled cannabinoids.

Methods: THC-COOH was purchased from Cerilliant (Round Rock, TX, USA). THC-COOH-spiked blank urine (100 µL) underwent alkaline hydrolysis followed by protein precipitation with acetonitrile and ICD. THC-COOH was derivatized with isopropyl-piperidine carboxylic acid hydrazide (IPPAH), while the internal standard was generated using the deuterated analogue IPPAH-d_6 in the presence of N,N,N′,N′-tetramethyl-O-(N-succinimidyl)uronium hexafluorophosphate (HSTU). After derivatization, samples were purified using monolithic C18 spin columns (MonoSpin C18, GL Sciences, Tokyo, Japan) prior to analysis.

LC–MS/MS analysis was performed using a Shimadzu Nexera XS UHPLC system coupled to an LCMS-8050 triple quadrupole mass spectrometer equipped with an electrospray ionization (ESI) source operated in positive-ion mode. Chromatographic separation was achieved on a Raptor ARC-C18 column (100 × 3.0 mm, 1.8 µm) maintained at 40 °C with a flow rate of 0.4 mL/min. The mobile phases consisted of (A) water containing 10 mM ammonium formate and 0.1% formic acid and (B) methanol containing 10 mM ammonium formate and 0.1% formic acid. The gradient started at 70% B, increased linearly to 85% B, and returned to initial conditions with a total run time of 20 min. Detection was performed in multiple reaction monitoring (MRM) mode.

Results: The derivatized analyte (THC-COOH-IPPAH) and isotope-coded internal standard (THC-COOH-IPPAH-d_6) exhibited identical chromatographic retention and similar ionization behavior, supporting reliable isotope-coded quantification. Calibration curves were linear from 1–500 ng/mL ($r^2 > 0.999$). Intra- and inter-day accuracy ranged from 81.8–108.6%, with precision below 10%. Internal-standard–normalized matrix factors ranged from 97–109% with low variability, indicating effective compensation for matrix effects. Extraction recovery ranged from 85.6–107.1%. Derivatized samples were stable for at least 72 h in the autosampler with no detectable H/D exchange.

Discussion: The isotope-coded derivatization strategy provides quantitative performance comparable to conventional isotope dilution while eliminating reliance on commercially supplied isotope-labeled THC-COOH standards. Because the internal standard is generated through derivatization of the analyte using readily synthesized reagents, this approach may facilitate implementation of quantitative cannabinoid testing in laboratories where labeled standards are difficult to obtain. The method also highlights the potential of ICD strategies for LC–MS/MS quantification of other drugs and metabolites containing carboxylic acid functionalities in forensic toxicology.

Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

O-027

Material Matters: Evaluating Polymer 96-Well Plates to Reduce DAMGO Loss in LC-MS/MS-Based in Vitro μ-Opioid Structure Affinity Assay

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Abstract

Introduction: Opioids are known to cause respiratory depression, a potentially fatal condition mediated by μ-opioid receptor (MOR) agonism. Therefore, assessing the binding affinity at this receptor is crucial to identify dangerous substances. Radioactivity-based competitive binding affinity assays have been commonly used for these types of studies, with the synthetic tetrapeptide [D-Ala[2], N-MePhe[4], Gly-ol]-enkephalin (DAMGO) being one of the most commonly used competing references. In these assays, a reference is competing with a test substrate for the binding site and the signal of the reference compound is measured. Recently, a competitive liquid chromatography tandem mass spectrometry (LC-MS/MS) receptor binding assay has been presented by Volz and Moosmann for the determination of the binding affinity at MOR using DAMGO as the competing reference. A great challenge in the development of appropriate assay methods is the choice of laboratory consumables, since they can interact with the investigated analytes. In fact, non-specific adsorption (NSA) at plastic surfaces has been reported for various types of peptides but, to the best of our knowledge, not for the here utilized DAMGO. However, we encountered this phenomenon while implementing a previously published competitive LC-MS/MS based receptor affinity assay.

Objectives: The purpose of this study was to identify 96-well plates appropriate for a competitive LC-MS/MS receptor binding assay using DAMGO.

Methods: Eight different plastic plates from six manufacturers were tested under two different incubation conditions. In brief, an aqueous DAMGO solution (0.5 ng/mL) was sequentially transferred across multiple wells up to six times. To simulate assay conditions, the plates were incubated at 27°C for 1 hour or at 4°C for 24 hours, so as to assess how NSA was affected by the temperature and incubation time. Signal loss was determined against two types of controls which were concomitantly measured: one prepared in glass vials and one in the respective tested plastic plates. Both controls were never transferred. Measurement of DAMGO was performed using LC-MS/MS.

Results: Signal loss remained under 5% compared to either type of control only for one of the plates tested (Thermo Fisher (60180-P133)). This was observed regardless of the incubation type and number of transfers. For all other plates but one, a significant decrease in the signal of DAMGO was observed, with NSA worsening as the number of transfers increased. However, for the coated microtiter plates from Greiner Bio-One (655901) significant signal enhancement was observed, which may also lead to issues during quantification.

Discussion: This study shows the importance of testing for and identifying appropriate laboratory products early into the development of assay methods, as NSA can result in significant underestimation of analyte concentrations during quantification. The variation between the performances of each plate tested highlights that, if composed of the same material, laboratory consumables may still behave differently under experimental conditions. For the case of polymeric materials, the temperature, additives and manufacturing may significantly affect the surface characteristics of the final product, leading to different degrees of NSA.

Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

O-028

Overcoming Conversion, Chromatography, and Stability Challenges in the Analysis of Mitragynine-Related Compounds

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Abstract

Introduction: Kratom (*Mitragyna speciosa*) prevalence has been increasing due to its widespread availability in gas stations, smoke/vape shops, and on the internet. Mitragynine, its primary alkaloid, is metabolized to 7-hydroxymitragynine and is further metabolized to mitragynine pseudoindoxyl. Recently, 7-hydroxymitragynine, mitragynine pseudoindoxyl, and dihydro-7-hydroxymitragynine, a synthetic analog of 7-hydroxymitragynine, have appeared in products sold in a variety of formulations including tablets and liquids. Ingestion of these highly potent mu-opioid receptor agonists may result in opioid toxicity. Thus, shifting novel psychoactive substance (NPS) markets result in the need to reassess existing analytical methods and adapt to these changing analytical targets to improve toxicological case interpretation.

Objectives: This presentation describes the analytical challenges faced during the development and validation of a qualitative method to confirm the presence of 7-hydroxymitragynine, dihydro-7-hydroxymitragynine, mitragynine, and mitragynine pseudoindoxyl in blood and urine.

Methods: Various sample preparation techniques were evaluated for analyte recovery and stability. Instrumental analysis was performed on an Agilent 1290 Infinity II liquid chromatograph equipped with a Restek Raptor ARC-18 (2.7 µm, 100 x 3.0 mm) coupled to an Ultivo triple quadrupole mass spectrometer (LC-MS/MS). This method was validated qualitatively following ANSI/ASB Standard 036. Different storage conditions (4°C and -20°C) were investigated using fortified sodium fluoride-preserved blood and unpreserved urine to assess analyte stability over the course of 3 months. Stability experiments were evaluated with a six-point calibration curve from 1-100 ng/mL and controls at 3, 40, and 80 ng/mL to characterize quantitative deviations.

Results: An LC-MS/MS method was validated to confirm the presence of 7-hydroxymitragynine, dihydro-7-hydroxymitragynine, mitragynine, and mitragynine pseudoindoxyl in blood and urine, while ensuring separation of mitragynine diastereomers. A minor isotopic peak from paynantheine, a kratom alkaloid, was unable to be separated from mitragynine. In addition, 7-hydroxyspeciogynine was not chromatographically separated from its isomer 7-hydroxymitragynine. Nearly all neat commercial standards contained trace amounts of other target analytes as impurities. The existing mitragynine solid phase extraction method demonstrated notable mitragynine formation from dihydro-7-hydroxymitragynine in both blood and urine. This conversion was reduced when using a protein crash for blood. Ultimately, a liquid-liquid extraction method mitigated conversion while maintaining desired limits of detection at 1 ng/mL. Results of an ongoing stability study will be presented.

Discussion: With the increasing prevalence of mitragynine-related compounds available to consumers, there is a need to accurately identify these analytes in toxicological specimens. To address this, a method was successfully validated for the qualitative confirmation of these analytes. Confirmation methods must reduce or eliminate in vitro analyte conversion to

ensure accurate reporting and proper toxicological interpretation in light of new NPS products available on the market. In addition, storage conditions have the potential to influence analyte stability and should be taken into consideration, particularly in suspected cases involving mitragynine-related alkaloids.

Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

O-029

Misidentification of TFMPP in Forensic Toxicology: A Cautionary Case Involving Aripiprazole Metabolism

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Abstract

Introduction: Third-generation antipsychotics, including aripiprazole, are widely prescribed for the treatment of psychiatric disorders. Aripiprazole undergoes extensive hepatic metabolism, producing several metabolites, among which dehydroaripiprazole is routinely monitored in toxicological analyses. However, lesser-known metabolites, such as 1-(2,3-dichlorophenyl) piperazine (DCPP) may present analytical challenges. Piperazine derivatives, including 3-trifluoromethylphenylpiperazine (TFMPP), have previously been encountered as recreational substances. Due to structural similarities and comparable fragmentation patterns, this compound may lead to analytical interferences.

Objectives: The aim of this study was to investigate the potential misidentification of TFMPP in biological samples from aripiprazole-positive cases and to evaluate analytical strategies enabling reliable differentiation between TFMPP and structurally related metabolite, DCPP.

Methods: Postmortem biological samples were subjected to liquid–liquid extraction using ethyl acetate under alkaline conditions (pH 9). Toxicological screening and confirmatory analyses were performed using ultra-high-performance liquid chromatography coupled with triple quadrupole tandem mass spectrometry (UHPLC–MS/MS). Analyses were conducted in multiple reaction monitoring (MRM), product ion scan, and full scan modes under positive electrospray ionization. Additional experiments included modification of chromatographic conditions and evaluation of alternative MRM transitions to improve compound discrimination.

Results: Initial toxicological screening indicated the presence of 'TFMPP' in both blood and vitreous humor samples from a fatal case involving multiple traumatic injuries. However, subsequent product ion scan analyses excluded the presence of TFMPP. Detailed spectral evaluation and database comparison confirmed that the detected signal corresponded to DCPP, a metabolite of aripiprazole. Both compounds exhibited similar precursor ions (m/z 231) and shared fragment ions, explaining the observed analytical interference. Furthermore, coelution under routine chromatographic conditions contributed to the misidentification. Notably, similar analytical findings were observed in multiple aripiprazole-positive cases, suggesting that this issue is not isolated. The presence of characteristic chlorine isotopic patterns (9:6:1) supported the identification of DCPP. Adjustment of MRM transitions and chromatographic conditions enabled improved differentiation between the two compounds.

Discussion: This study demonstrates that misidentification of TFMPP may occur in aripiprazole-positive cases due to coelution and overlapping fragmentation patterns with DCPP. Such misinterpretation carries significant forensic risk, as false-positive identification of a recreational substance may lead to incorrect conclusions in both clinical and legal contexts. The results underscore the importance of comprehensive analytical strategies, including the use of certified reference standards, expanded metabolite monitoring, and verification using alternative acquisition modes or chromatographic conditions. Particular attention should be given to isotopic patterns and less commonly monitored metabolites. The findings highlight the necessity for critical evaluation of unexpected toxicological results, especially when they are inconsistent with case history.

Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

O-030

The Meth Mimic: Unmasking N-Isopropylbenzylamine in Forensic Analysis

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Abstract

Introduction: Accurate identification of methamphetamine in forensic toxicology is essential for clinical interpretation and harm-reduction advice. However, structural isomers and adulterants can complicate analysis. N-isopropylbenzylamine is an isomer of methamphetamine that has been reported internationally as an adulterant or as a complete substitute of methamphetamine in seized drug samples. Due to its structural similarity and overlapping mass spectrometric fragments, N-isopropylbenzylamine can be mistaken for methamphetamine in certain analytical methods.

Objectives: This study describes the investigation of a coroner's toxicology case in which an apparent methamphetamine result was subsequently identified as N-isopropylbenzylamine, highlighting analytical challenges and the importance of confirmatory testing.

Methods: A biological sample submitted for toxicological analysis underwent protein precipitation with acetonitrile. The supernatant was evaporated to dryness and reconstituted in 10% acetonitrile in water.

Initial analysis was performed using an Agilent 1100 HPLC coupled to an API 4000 QTRAP mass spectrometer with a Supelco Discover HS F5 column under isocratic conditions (50% acetonitrile with 0.1% formic acid; 8-minute run). Methamphetamine and methamphetamine-d5 were monitored at m/z 150 and 155, with confirmation using the m/z 91 fragment ion.

Further analysis was conducted using a Thermo Vanquish Flex UHPLC system coupled to an Exploris 120 Orbitrap high-resolution mass spectrometer (HR-MS) with a Thermo Accucore Phenyl Hexyl column with a gradient of 1% to 99% organic over 10 minutes, held for 1.5 mins then re-equilibrated for a total run time 15.5 minutes. Accurate mass measurements (±5 ppm), retention times, and MS/MS fragmentation spectra were evaluated. A reference standard of N-isopropylbenzylamine was subsequently purchased and analysed under identical HR-MS conditions.

Results: On the API 4000 system, a peak consistent with methamphetamine was observed co-eluting with the methamphetamine-d5 internal standard. As this method relied on a single confirming fragment (m/z 91), the result initially appeared consistent with methamphetamine.

However, HR-MS analysis demonstrated that the peak of interest eluted at 2.98 minutes and did not co-elute with the methamphetamine-d5 internal standard which was running at 3.33 minutes. Although spectral library matching suggested methamphetamine, detailed inspection revealed the presence of the m/z 91.0542 fragment but absence of the characteristic methamphetamine fragment at m/z 119.0855.

Accurate mass data (m/z 150.1278 ± 5 ppm) and fragmentation patterns were consistent with N-isopropylbenzylamine. Analysis of a purchased N-isopropylbenzylamine reference standard confirmed both retention time and MS/MS spectral match, with the presence of the m/z 91.0542 fragment present and absence of m/z 119.0855.

Discussion: This case demonstrates the potential for N-isopropylbenzylamine to mimic methamphetamine in targeted LC–MS/MS methods that rely on limited fragmentation data and insufficient chromatographic separation. Use of high-resolution mass spectrometry, multiple fragment ions, and retention time comparison with internal standards was critical in avoiding misidentification.

Given the increasing reports of N-isopropylbenzylamine in drug seizures and its unclear toxicity profile, accurate differentiation from methamphetamine is essential. Laboratories should ensure confirmatory methods incorporate multiple diagnostic fragments and adequate chromatographic resolution to reduce the risk of false-positive identification.

Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

O-031

Characterization of Adduct Formation by Co-Eluting Compounds During LC-MS Ionization

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Abstract

Introduction: Even though LC-MS techniques are widely implemented in toxicological analysis and many other analytical disciplines, certain aspects of the underlying ionization processes remain insufficiently understood. This knowledge gap complicates the interpretation of analytical results, as matrix effects — such as ion suppression and enhancement — can significantly influence quantitative results. Recently, a previously undescribed adduct formation was observed under negative ionization conditions for the diuretic hydrochlorothiazide when co-eluting with the endogenous biomolecules hippuric acid and indoxyl sulfate (Triebel et al., 2025). This finding highlights the need for further investigation of such phenomena.

Objectives: This study further analyzed similar adduct formation processes involving the endogenous, human-specific biomolecule phenylacetyl-L-glutamine by employing positive electrospray ionization (ESI) and atmospheric-pressure chemical ionization (APCI). In addition to characterizing this phenomenon, the study evaluated the influence of analyte concentration, ionization technique and instrument design.

Methods: Systematic investigations either by gradient elution on a RP C18 column and/or by post column experiments were conducted using reference substances. In addition to assessing the dependence of adduct formation on the concentrations of both interacting partners, the influence of source design and ionization technique (ESI or APCI) was systematically examined. To this end, two different LC-MS/MS instruments (Thermo Fisher QExactive and Shimadzu 8050) were used.

Results: During LC-MS/MS urine analysis, adduct formation between phenylacetyl-L-glutamine and baclofen was observed. Further analysis of structurally related compounds revealed that the antiepileptic drugs pregabalin and gabapentin can also form adducts with phenylacetyl-L-glutamine. Adduct formation was observed using two different LC-MS/MS instruments. Additionally, adduct formation occurred under ESI and APCI conditions and showed a concentration-dependent relationship for both interacting components. In contrast to baclofen, the corresponding adducts of pregabalin and gabapentin were detectable in authentic serum samples and calibrators using the Shimadzu 8050 platform in combination with the RECIPE TDM 200 kit. Serum phenylacetyl-L-glutamine concentrations varied considerably, with authentic samples exhibiting levels approximately two- to 30-fold higher than calibrators. The peak area of the formed adduct accounted for up to 10% of the antiepileptic drug signal. Adduct formation with phenylacetyl-glutamine was also observed for the employed deuterated standards at a comparable level. These findings suggest that the use of a structurally similar internal standard with a retention time close to that of the analyte can largely compensate for the observed adduct formation.

Discussion: This study underscores the complexity of ionization processes within biological matrices, describing adduct formation by co-eluting compounds. These findings demonstrate that adduct formation between co-eluting compounds is a reproducible and concentration-dependent phenomenon in biological matrices. The observation of adduct formation across different ionization techniques and instruments highlights its general relevance. Those adduct formation processes may cause bias in quantitative LC-MS methods, particularly when no stable-isotope-labelled internal standard is used. This research forms the basis of a more comprehensive understanding of the processes involved in ionization, which are critical for interpreting analytical data.

Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

O-032

Drift You Didn't Know You Had: Development and Validation of an Improved HS-GC-FID Method for Quantitative Ethanol Determination Across Matrices in Forensic Toxicology

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Abstract

Introduction: Headspace gas chromatography with flame-ionization detection (HS-GC-FID) is widely used for quantitative ethanol determination in forensic toxicology. Although generally considered robust, routine workflows often rely on small batch sizes, masking subtle equilibrium-related effects. During method scale-up, our laboratory observed a systematic positive drift in calculated ethanol concentrations when analysing batches exceeding ten vials, reaching up to +10% over a series of 100 samples — an effect that has direct implications on result accuracy.

Objectives: The study aimed to (1) identify the cause of the observed positive drift in ethanol quantification during large-batch HS-GC-FID analysis, (2) develop a strategy to eliminate this drift, and (3) validate an improved method for accurate quantification across relevant forensic matrices.

Methods: Drift patterns were evaluated across batches of up to 120 vials, with particular attention to headspace–liquid equilibrium shifts occurring during autosampler queueing. To counteract equilibrium drift, saturated NaCl solution was added to each vial to induce "salting out" and stabilize gas-phase partitioning.

Resulting changes in analyte and internal standard (ISTD) recovery were assessed across matrices including water, urine, plasma, whole blood and vitreous humor.

Matrix-dependent recovery effects were mitigated by reducing sample volume (to 50 µL), preparing the ISTD (t-butanol, selected for its suitability in post-mortem casework) in saturated NaCl, extending vial equilibration time to 15 min, and enabling vial shaking in the headspace autosampler.

Method validation followed established forensic toxicology guidelines.

Results: Using the conventional HS-GC-FID procedure, batches exceeding approximately 10 vials showed a measurable positive drift in ethanol response. Critically, the drift had a greater effect on ethanol than on the ISTD, preventing the internal standard from compensating and causing a progressive overestimation of calculated ethanol concentrations. This effect was attributed to continued phase equilibration inside sealed vials during autosampler queueing.

To shift partitioning toward the gas phase, saturated sodium chloride solution was introduced during sample preparation. Addition of saturated NaCl improved overall recovery via salting-out but introduced matrix-dependent variation in both analyte and internal standard recovery, complicating universal calibration across different matrices. By reducing the sample volume and optimizing headspace autosampler parameters, matrix effects were substantially reduced, allowing accurate quantification in different matrices while maintaining stable response over extended batch sizes.

Validation demonstrated: linearity across 0.2–3.0 g/L ($R^2 > 0.995$); within-run and between-run precision RSD < 6%, bias <4% and no significant carryover or robustness issues.

Discussion: The original method used in this study is representative of procedures widely described in peer-reviewed literature and instrument vendor application notes and therefore likely reflects routine practice in many forensic laboratories. This makes the observed drift not an isolated finding, but a relevant risk across the field.

The salting-out approach, combined with optimized instrumental parameters, resolves both the equilibrium drift and matrix dependency issues. The validated method is suitable for high-throughput forensic casework across diverse biological matrices. These findings highlight the importance of large-batch validation studies and have implications for any forensic laboratory relying on conventional HS-GC-FID ethanol methods.

Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

O-033

Third Time's the Charm: Revalidation of an Alcohol Analysis Method

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Abstract

Introduction: Replacing instrumentation for an analytical method requires revalidation to ensure that the method remains accurate, reliable, and fit for its intended use. A procedure to quantitate and confirm ethanol was optimized and revalidated using headspace (HS) gas chromatography (GC) with simultaneous flame ionization (FID) and mass spectrometry detection (MS). According to the ANSI/ASB 036 standard for method validation, only three replicates are required per batch to calculate within-run precision for each concentration level. In this revalidation, an additional repeatability and drift study was conducted. When analyzing 43 replicates of a 0.300 g/dL standard, there was unexpected variability in precision results that did not meet Florida Administrative Code (FAC). After extensive troubleshooting, including changing the autosampler, the validation was prolonged to become a two-year project.

Objectives: This presentation covers the problems encountered, how they were identified, troubleshooting, and solutions that were implemented.

Methods: One hundred microliters of blood, urine, or aqueous matrix was diluted with 1 mL of n-propanol internal standard solution using a Hamilton Diluter-Dispenser and analyzed by HS-GC-FID/MS. The revalidation was completed based on the lab's validation requirements and the ANSI/ASB Standard 036 but does not fully comply with the standard's requirements, such as not using matrix-matched fortified samples.

Results: The initial revalidation plan used an Agilent 8697 HS Sampler, 8890 GC and 5977B MS. During the drift experiment, 43 replicates at the 0.300 g/dL control level failed to obtain minimum and maximum results that met the FAC requirement to be within 0.01 g/dL. Even after limiting to 35 replicates, the difference between the maximum and minimum ranged from 0.0048 to 0.0111. Three previous validation studies on the older instrument showed better precision between 40 replicates at the same control level ranging from 0.0034 to 0.0064. Troubleshooting included changing the inlet split ratios, capillary flow technology splitter configuration, temperature program, vial equilibration time, etc. None of these parameter changes yielded acceptable results, so the autosampler was replaced with an Agilent PAL3 rail autosampler. The PAL3 yielded acceptable precision, however, had constant communication errors, failure of vial transportation, and errors with the agitator. These issues were not resolved despite several on-site visits from technicians. Therefore, the autosampler was replaced again with an Agilent 7697A HS autosampler. Acceptable precision was achieved and the validation was completed using the 7697A.

Discussion: Previous validation studies with an Agilent G1888 HS autosampler identified positive drift when analyzing 65 replicates, which was mitigated by limiting the number of vials for a batch. However, the positive drift observed with the 8697 autosampler led to unacceptable precision despite decreasing the number of replicates because concentrations spiked randomly at various points throughout the batch. It was not determined if the observed performance was due to a faulty unit or the design of the HS instrument. If the revalidation experiments had relied solely on ASB 036 and tested only three replicates in a batch, the precision issue would not have been

identified. This revalidation highlights the necessity of evaluating within-run repeatability and drift, extending beyond the ASB 036 requirements, for alcohol testing using HS autosamplers that use a sample loop design.

Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

O-034

Untargeted GC-MS Metabolomic Profiling of Oral Fluid From Psychoactive Drug-Positive Drivers: Exploratory Assessment of Ketamine-Associated Metabolic Alterations

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Abstract

Introduction: Driving under the influence (DUI) of psychoactive drugs remains a major public health concern. Ketamine has increasingly been reported in illicit drug markets and detected in drivers during roadside drug controls. Oral fluid (OF) is widely used in forensic toxicology due to its non-invasive collection and its ability to reflect recent drug use. Current forensic approaches focus on detecting parent drugs and their metabolites, while biochemical alterations associated with drug consumption remain poorly understood. Although metabolomics may provide complementary insights into metabolic changes related to drug exposure, studies investigating drugs of abuse in OF using this approach remain scarce.

Objectives: This study aimed to explore metabolomic alterations in OF from drug-positive drivers and evaluate whether metabolic profiles differ from drug-free controls using an untargeted GC-MS-based metabolomics approach.

Methods: OF samples were obtained from drivers subjected to roadside drug testing. Confirmatory analysis was performed using LC-MS/MS to determine ketamine and other drugs of abuse. Several comparison groups were included (n=15 per group): ketamine-positive drivers, drivers positive exclusively for cocaine, amphetamines, or THC, and drug-free controls. Untargeted metabolomic profiling was performed using GC-MS with two analytical strategies: derivatization-based analysis of polar metabolites including amino acids, sugars, and organic acids, and headspace solid-phase microextraction (HS-SPME) for the analysis of volatile organic compounds (VOCs). Data were processed and analyzed using multivariate (principal component analysis, PCA; partial least squares discriminant analysis, PLS-DA) and univariate statistical analyses, applying false discovery rate (FDR) correction.

Results: The volatile metabolite profile did not show clear discrimination between drug-free controls and drug-positive groups in multivariate analyses, suggesting limited discriminatory power of volatile compounds. PLS-DA models obtained from the analysis of derivatized metabolites showed separation between drug-positive samples and drug-free controls ($Q^2>0.5$). Similar metabolic alterations were observed across the evaluated drug groups, although these changes appeared more pronounced in ketamine-positive samples. When ketamine-positive drivers were specifically compared with controls, the PLS-DA model showed improved discrimination compared with the other drug-positive groups ($Q^2=0.67$; permutation test $p=0.013$). Combined multivariate and univariate analyses identified several metabolites significantly increased across drug-positive samples, including glutamate, aspartate, glutamine, pyroglutamate,

proline, serine, valine, and isoleucine, suggesting alterations in amino acid metabolism. Although none of these metabolites were specific to a particular drug group, multivariate analysis showed that only ketamine-positive drivers could be clearly discriminated from controls.

Discussion: This study suggests that the OF metabolic profile of drug-positive drivers is characterized by alterations in amino acid metabolism. Several metabolites identified are linked to glutamate metabolism and nitrogen balance, pathways involved in neurotransmission and cellular regulation. Psychoactive drugs may perturb these systems through effects on neurotransmitter signaling, oxidative stress and metabolic homeostasis; in particular, ketamine acts as an NMDA receptor antagonist, which may explain glutamate-related alterations. Although metabolomic studies investigating drugs of abuse in OF remain scarce, similar perturbations have been reported in urine and plasma metabolomic studies on drug exposure. Together, these findings provide preliminary evidence that OF metabolomics can detect metabolic alterations associated with drug consumption and may complement conventional toxicological analysis in DUI investigations.

Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

O-035

Breaking the Law, Not the Habit: A Decade of Methamphetamine Related Driving While Impaired (DWI) Casework in New Mexico

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Abstract

Introduction: Methamphetamine is a potent and addictive central nervous system stimulant with high potential for abuse. Its history of use in New Mexico is notorious, and it continues to be a prominent drug in DWI casework throughout the state.

Objectives: This study retrospectively examined trends across a ten-year period for methamphetamine-related DWI cases across the state of New Mexico. Scant literature related to high blood concentrations of methamphetamine in impaired driving casework and characteristic effects at such concentrations is noted.

Methods: Toxicology data from impaired driving cases in the State of New Mexico between 2015–2024 were compiled. Cases that underwent drug screening were then filtered by reportable methamphetamine, and concentrations were evaluated. Statistical analysis and trend assessment for this time period were completed using Excel.

Results: In New Mexico, between 2015-2024, 6,942 driving related cases underwent drug screening. Of those, 29.5% (2,045) had a confirmed and reported methamphetamine result. The average methamphetamine concentration was 0.36 mg/L, the median was 0.27 mg/L, the mode was 0.10 mg/L, and the maximum was 4.7 mg/L. Three cases with incident reports were identified to have methamphetamine concentrations > 2.0 mg/L.

Case Study 1: Fluoxetine: Present, Methamphetamine: 2.6 mg/L, Amphetamine: 0.18 mg/L

A 35-year-old male was observed weaving and nearly struck a police cruiser. Upon stop, the driver appeared drowsy with droopy eyelids and bloodshot eyes. The driver admitted to “nodding off” while driving and to taking clonazepam. Two grams of a crystalline substance and a pipe were found on person. Standard Field Sobriety Tests (SFSTs) revealed six clues on the Horizontal Gaze Nystagmus, four clues on the Walk and Turn, and two clues on the One Leg Stand. Poor performance on alternative tests was also noted.

Case Study 2: Ethanol: 0.01 g/100mL, THC: 2.0 ng/mL, Carboxy THC: 12.9 ng/mL, Amphetamine: <0.05 mg/L, Methamphetamine: 3.9 mg/L

A 29-year-old male was evading police at a high rate of speed, almost struck other vehicles, and was eventually stopped using spike strips. The driver threw a pistol and pills out of the vehicle while being pursued and was holding an open container upon apprehension. SFSTs were not conducted given violent demeanor of suspect.

Case Study 3: Lorazepam, Promethazine, Morphine: Present, Amphetamine: 0.07 mg/L, Methamphetamine: 4.7 mg/L

Police responded to the site of a crash involving fatalities. The driver, a 51-year-old male, was stabbing the hole where his airbag had deployed from the steering wheel and telling police, “I’ll kill you”. The driver was sedated at the hospital due to his behavior.

Discussion: Findings indicate a significant proportion (29.5%) of New Mexico DWI casework screened for drugs contained methamphetamine. Concentration irregularity was obvious, with many cases surpassing thresholds associated with overdose risk. The prevalence and high concentrations of methamphetamine in DWI casework, coupled with variability of its characteristic effects, present challenges for law enforcement and testing laboratories by complicating impairment assessment, toxicological interpretation, and the reliable quantification of methamphetamine across a broad concentration range. Given its historical prevalence and despite evolving drug-related driving trends, methamphetamine continues to impose a proportionally significant burden on New Mexico.

Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

O-036

On-Site Testing, LC-MS/MS and LC-HRMS in DUID Investigations: A Head-to-Head Analytical Performance Study From the French NPS ALERT (New Psychoactive Substances Analysis, Legal Enforcement, and Road Traffic) Study

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Abstract

Introduction: Driving Under the Influence of Drugs (DUID) is a significant public safety concern. Beyond conventional drugs of abuse, NPS and various psychotropic medications can severely impair driving performance. These substances are frequently omitted from routine toxicological screenings in France. The NPS-ALERT study is a national epidemiological study conducted in France in 2025, aimed at assessing the prevalence of emerging psychoactive substances in drivers' oral fluid (OF) samples collected by law enforcement using the Dräger DrugCheck 3000 device.

Objectives: This ancillary study primarily aimed to compare the analytical performance of LC-MS/MS and LC-HRMS approaches in order to harmonize toxicological protocols across French laboratories, and secondarily to evaluate the diagnostic accuracy and operational reliability of the Dräger DrugCheck 3000 under real-world conditions.

Methods: Six forensic laboratories across the national territory participated using a harmonized extraction protocol and standardized Limits of Detection (LODs). These limits were set at 1 ng/mL for THC and 10 ng/mL for ketamine, cocaine, opiates, amphetamines, and selected NPS representing the primary pharmacological classes (x-MMC, MDMB-4-en-PINACA, etizolam, metonitazene, dimethyltryptamine). Detection rates were compared between LC-MS/MS and LC-HRMS. The diagnostic performance of the Dräger DrugCheck 3000 was assessed against mass spectrometry as a gold standard method using nationwide real-world samples. An experimental evaluation was conducted to assess the analytical performance, specifically sensitivity, of the Dräger DrugCheck 3000. Authentic drug-free OF was spiked with cocaine, THC, methamphetamine, amphetamine, and morphine at four concentrations (1×, 2×, 5×, and 10× the cutoff), utilizing six replicates per concentration.

Data analysis was conducted using Claude Code (Anthropic, 2026), an agentic command-line interface (CLI) tool integrated within the visual studio code development environment.

Results: This study included 2553 OF samples, of which 2328 were analyzed with both LC-MS/MS and LC-HRMS. LC-MS/MS enabled the detection of 1,657 parent drugs and metabolites, while LC-HRMS identified 1,384 compounds. Regarding THC, LC-MS/MS demonstrated superior performance with 317 identifications compared to 287 for LC-HRMS. For MDMA, LC-MS/MS also outperformed LC-HRMS, yielding 36 versus 27 positive identifications, respectively. For cocaine, LC-MS/MS and LC-HRMS demonstrated comparable performance (149 versus 147 identifications, respectively. For NPS (X-MMC, 2F-DCK, O-PCE), LC-HRMS identified seven instances, whereas LC-MS/MS detected only three. Of the 2,328 OF samples analyzed for conventional drugs of abuse (DoA), the Dräger DrugCheck 3000 device achieved an overall screening-to-confirmation concordance of 83.1%, with a specificity of 96.8% and a sensitivity of 58.1%, corresponding to 345 false negative results. THC detection by the Dräger device was markedly limited: positive results were obtained only at concentrations five times above the regulated screening cutoff (15 ng/mL), and in merely 1 out of 6 replicates (16.7%). For cocaine, detection was achieved at twice the regulated screening cutoff (10 ng/mL).

Discussion: While LC-MS/MS is highly effective for the targeted screening of conventional DoA, LC-HRMS remains essential for the detection of NPS. Furthermore, the performance of the Dräger device is concerning; beyond its failure to detect several common substances, the high incidence of false-negative results is particularly problematic. These findings suggest that a significant number of impaired drivers may go undetected during routine law enforcement roadside checks.

Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

O-037

Three DUI Case Studies of Ketamine Only Cases in the State of Colorado

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Abstract

Introduction: Ketamine is a dissociative anesthetic used medicinally to induce and maintain anesthesia, sedation, pain relief, and has also been used clinically as an antidepressant. In Colorado, Ketamine administration by Emergency Medica Services (EMS) is limited by some agencies. Additionally, Ketamine clinics are present throughout the state for the treatment of treatment-resistant depressive disorders. Ketamine may also be used recreationally and illicitly for its dissociative anesthetic and hallucinogenic effects. Since October of 2024, the Colorado Bureau of Investigation (CBI) Toxicology has screened all driving under the influence (DUI) cases submitted for Ketamine by Enzyme Linked Immunosorbent Assay (ELISA) due to suspected increase in prevalence.

Objectives: To review three DUI cases, that tested positive for Ketamine and Norketamine only, submitted to CBI in 2025. This presentation will outline and compare driving performance, subject behavior, symptoms, and indicators of impairment in three real world cases.

Methods: Blood samples submitted to CBI for DUI testing undergo analysis for Ethanol/Volatiles and a preliminary drug screen, via ELISA. The ELISA screen includes 15 different drug classes, including Ketamine (Cutoff – Ketamine 10 ng/mL). Cases which screen Ketamine positive are subsequently confirmed via outsourcing to National Medical Services (NMS) Labs.

Results: In 2025, CBI Toxicology completed 10,048 cases, of which 112 screened positive for Ketamine. Of these 112 cases, 7 reported only Ketamine. Police reports for three of these cases were obtained and reviewed.

The cases reviewed were single vehicle traffic stops that did not involve a crash. Ketamine was not medically administered in any of the three cases. In each case the subject voluntarily participated in roadside maneuvers and was subsequently arrested for DUI. For all three cases, Ketamine and Norketamine were the only positive findings. Table 1 outlines the demographic information and results for each case.

Table1: Demographic information and results for three Ketamine only DUI Cases.

Case	Subject details	Time Between stop and draw (minutes)	Ketamine (ng/mL)	Norketamine (ng/mL)
1	33 y/o M	127	74	220
2	38 y/o F	69	490	1,000
3	27 y/o F	163	830	900

Discussion: The same roadside maneuvers (horizontal gaze nystagmus (HGN), walk and turn, and one leg stand (OLS)) were conducted in all three cases. The roadsides were stopped during the walk and turn test for case 3 due to the inability of the subject to follow directions. All three subjects presented with bloodshot and watery eyes, slowed movements, horizontal gaze nystagmus, and did not seem to be fully coherent. The subjects also presented with indicators of impairment including, impaired divided attention, lane weaving, diminished balance and coordination, lack of concentration, and difficulty following directions. The review of these three cases shows that Ketamine, when taken by itself, can cause impairment in individuals at various concentrations. With its increase in prevalence, screening for Ketamine can provide important context for DUI investigations as an additional source of intoxication.

Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

O-038

Evaluation of the Draeger DrugCheck 3000 (DDC3000) as a Roadside Oral Fluid Screening Device Including Fentanyl Detection and Cross-Reactivity Assessment

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Abstract

Introduction: With the rise in drug-impaired driving in the United States, law enforcement agencies are increasingly relying on roadside oral fluid (OF) devices to detect drug use among drivers. These devices improve roadway safety by enabling rapid identification of potential impairment, helping reduce drug-related crashes and fatalities. Alabama has approved several OF devices for statewide use, including the Draeger DT5000, Abbott SoToxa, Randox MultiStat, and Securetec DrugWipe® S. The Draeger DrugCheck 3000 (DDC3000) is among the first roadside OF devices to incorporate fentanyl detection while simultaneously screening for multiple additional drug classes.

Objectives: This study evaluated the analytical performance, real-world applicability, and cross-reactivity profile of the DDC3000 as a roadside OF screening device.

Methods: The DDC3000 cartridge tests for eight drugs or drug classes: cocaine, Δ^9-tetrahydrocannabinol (THC), opiates, oxycodone, amphetamine, methamphetamine, benzodiazepines, and fentanyl.

In Phase I, simulated samples were prepared using pooled drug-free OF spiked at 50% below cutoff, at cutoff, and 50% above cutoff. Each level was tested in replicates (n=10 per concentration). Performance metrics including sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and accuracy were calculated. Eleven negative controls and two fentanyl-positive controls were included. Results were compared to confirmatory analysis by LC/MS/MS (Agilent Ultivo).

Cross-reactivity studies evaluated novel cannabinoids (Δ8-THC, Δ10-THC isomers, THC-O, THC-P, HHC, CBD) and fentanyl-related compounds (carfentanil, xylazine, para-fluorofentanyl, cychlorphine).

In Phase II, authentic samples were collected from 51 volunteers enrolled in rehabilitation programs in Jacksonville, Florida. OF specimens were collected using Quantisal devices and analyzed via LC/MS/MS to assess real-world performance.

Results: Simulated samples demonstrated excellent performance across all targets. Sensitivity, specificity, and accuracy reached 100%, with NPV at 100% and PPV ranging from 96–100%. Fentanyl achieved 100% across all performance metrics, indicating strong detection capability.

In authentic samples, performance for THC, cocaine, amphetamine, methamphetamine, and fentanyl exceeded 80% across all evaluated parameters. No positive authentic samples were collected for oxycodone, opiates, or benzodiazepines; therefore, performance for these analytes could not be assessed. Overall performance across all target analytes in simulated samples exceeded 94%.

Cross-reactivity was observed among several novel cannabinoids and opioids at varying concentrations. Δ8-THC and THC-P were cross-reactive at 10 ng/mL. THC-O and select Δ10-THC and HHC isomers demonstrated cross-reactivity at 100 ng/mL, while others required higher concentrations (500 ng/mL). CBD showed cross-reactivity only at 10,000 ng/mL. Para-fluorofentanyl demonstrated cross-reactivity at 5 ng/mL. Cychlorphine showed no cross-reactivity up to 2,000 ng/mL.

Discussion: This study supports the use of roadside OF testing as a valuable tool in DUI investigations. The DDC3000 demonstrated strong analytical performance as a roadside OF screening device across multiple drug classes, including fentanyl. Cross-reactivity findings further characterized device specificity and potential analytical limitations associated with emerging novel psychoactive substances.

Overall, the DDC3000 appears fit for purpose as a roadside OF screening device and may assist law enforcement agencies in improving detection of drug-impaired drivers and enhancing roadway safety.

Disclosure

Yes, I, or a member of my immediate family, has a financial interest to disclose.

Conflict of Interest

Honoraria/Expenses.

O-039

A Retrospective Evaluation of Oral Fluid DUID Casework Data in New York State

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Abstract

Introduction: Oral fluid has become an increasingly desirable testing matrix for driving under the influence of drugs (DUID) investigations. Oral fluid provides several advantages, including a rapid simple collection process relative to blood, and a detection window proximal to the time of drug use. The National Safety Council's Alcohol, Drug, and Impairment Division (NSC-ADID) has established analyte scope and sensitivity recommendations for testing oral fluid specimens in DUID investigations, which are based on drug prevalence and appropriate detection windows. In September 2023, the New York State Police implemented an evidential oral fluid program which meets or exceeds 2021 NSC-ADID Tier I recommendations.

Objectives: The objective of this review was to evaluate drug prevalence in oral fluid specimens analyzed for Tier I analytes in DUID investigations. Intra-class positivity rates and drug/metabolite relationships were also examined.

Methods: A review of oral fluid casework from September 2023 – December 2025 was conducted. Analytical methods were validated to ANSI/ASB Standard 036 and provide qualitative results. Data were organized by drug class, and comparisons were made within and across classes.

Results: A total of 1,482 oral fluid cases were examined. Cannabinoids (specifically Δ^9-THC) demonstrated the highest prevalence at 55.8% (n=827), followed closely by stimulants at 55.5% (n=823). Narcotic analgesics were present in 36.5% (n=541), benzodiazepines in 15.8% (n=234), and miscellaneous depressants in 2.0% (n=30) of total cases. No drugs within the scope were detected in 7.8% (n=115) of cases. The most prevalent drug combination in positive cases (n=1,367) was cocaine/metabolites and Δ^9-THC (21.2%, n=290), followed by cocaine/metabolites and fentanyl (15.9%, n=218).

For cocaine/metabolites, benzoylecgonine was the most prevalent (95% positivity rate) and was detected along with cocaine in 87.6% of those cases, while cocaine-only cases demonstrated a low prevalence of 5%. Fentanyl was the most prevalent of the narcotic analgesics detected, with a positivity rate of 61.9% within that class. 6-acetylmorphine was present in 16.3% of narcotic analgesic samples, and 96.6% of those also had fentanyl. For amines, amphetamine and methamphetamine had similar prevalence at 85.2% and 82.0% respectively, and 67.9% of amines positive cases contained both analytes. Amphetamine was detected on its own in 17.3% of amines cases. Within the benzodiazepine class, alprazolam had the highest prevalence (48.7%) and was commonly detected with clonazepam and/or 7-aminoclonazepam (17.5% of total alprazolam cases). 7-aminoclonazepam was primarily detected without clonazepam, accounting for 70.3% of clonazepam/7-aminoclonazepam cases. Clonazepam was not detected alone in any specimens.

Discussion: This retrospective analysis of drug prevalence in oral fluid specimens offers a real-world insight on positivity rates and drug/metabolite relationships in impaired driving populations, which may not be accounted for in controlled dosing environments or other drug testing populations. Positivity rates presented here coincide with national DUID trends for stimulants, Δ^9-THC, alprazolam, and fentanyl. In contrast to national trends, 6-acetylmorphine prevalence was greater than most narcotic analgesics. This observation indicates that oral fluid is a valuable specimen for the detection of heroin use. The inclusion of metabolites in the analyte testing scope may also enhance detectability for some analytes, in particular cocaine and clonazepam.

Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

O-040

Trust the Signs? A Data-Driven Look at DRE Evaluations

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Abstract

Introduction: The Drug Recognition Expert (DRE) program remains the primary tool for evaluating drug-related impairment at the roadside, with prior studies supporting its reliability for traditional drugs. However, existing research does not directly compare DRE face-sheet observations with confirmed toxicology results. This leaves a critical gap in understanding how well field assessments align with substance use, particularly in cases involving polysubstance use and variable concentrations. Direct comparison of DRE evaluations with toxicology findings is essential to assess the accuracy and reliability of impairment determinations.

Objectives: This presentation will examine the relationship between DRE assessments and laboratory-confirmed toxicology results to measure the reliability of field impairment evaluations.

Methods: Data from DRE evaluations conducted between January 1, 2023, and December 31, 2024, were exported from the data warehouse, converted to a binary format in Excel, and linked to confirmed toxicology results. Evaluation indicators (e.g., blood pressure, pulse, horizontal gaze nystagmus) were analyzed to determine the proportion of cases in which observations aligned with expected patterns for each drug class. Analyses were limited to cases with confirmed toxicology for a single drug class only and were stratified by whether the DRE call for that class was positive or negative.

Results: For cannabis, 294 cases were confirmed positive for delta-9 tetrahydrocannabinol (THC) and/or its metabolites only, of which 278 were identified by the DRE as cannabis-related impairment. The most common indicators included bloodshot eyes (91%), eyelid tremors (75–83%), elevated systolic blood pressure (63%), increased pulse rate (57–61%), and dilated pupils in darkness (56%) and direct light (49%). Mean and median THC concentrations were 9.4 ng/mL and 7.2 ng/mL, respectively.

For CNS stimulants, 409 cases were toxicologically confirmed, with 345 including a corresponding DRE call. Among the 149 single-class cases, common indicators were bloodshot eyes (88%), eyelid tremors (68–79%), elevated systolic blood pressure (79%), increased pulse rate (69–72%), and slow reaction to light (76%). Methamphetamine and amphetamine were detected in more than half of the cases with mean concentrations of 422 ng/mL and 58 ng/mL, respectively.

In the narcotic analgesic category, 253 cases included a DRE opinion with confirmed toxicology, of which 43 were single-class cases. Although lower pulse rate and blood pressure are expected indicators, only 12–26% of cases showed decreased pulse across measurements, and 26% had blood pressure below the expected range.

Discussion: Published data on DRE performance indicators in the context of confirmed toxicology are limited. In these findings, cannabis and CNS stimulant cases showed strong alignment with expected indicators, while narcotic analgesic cases demonstrated weaker concordance, but was limited by the total number of cases. Interpretation is constrained by delays between DRE evaluation and sample collection, which may reduce detectable drug concentrations

and by variability in dose, tolerance, and physiology. Overall, these results highlight both the utility and limitations of DRE evaluations and the need for further integrated research.

Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

O-041

Gabapentin in Impaired Driving Casework in Dallas County From 2018 to 2025

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Abstract

Introduction: Gabapentin is an approved treatment for epilepsy and neuropathic pain but due to a relatively minimal side effect profile at therapeutic doses it is also prescribed for numerous off-label uses. Due to an increasing number of prescriptions, the misuse and abuse of gabapentin has also increased. The increase in use of gabapentin has impacted its prevalence in forensic toxicology casework. In 2025, the National Safety Council Alcohol, Drugs, and Impairment Division (NSC-ADID) promoted gabapentin to Tier I due to increased reporting by laboratories across the United States. To determine the impact on driving while intoxicated (DWI) cases in Dallas County and the surrounding areas, this study reviewed DWI cases positive for gabapentin submitted to the Dallas County Southwestern Institute of Forensic Sciences (SWIFS) from January 2018 to December 2025.

At SWIFS, gabapentin was reported qualitatively from a comprehensive drug screen by liquid chromatography quadrupole time of flight mass spectrometry (LC-QTOF-MS) from 2018 to late 2022. In August 2022, gabapentin was added to an existing quantitative confirmation method by liquid chromatography tandem mass spectrometry (LC-MS/MS) with a reporting range of 200.0 to 20,000.0 ng/mL.

Objectives: To assess the prevalence of gabapentin use in Dallas County and surrounding areas from 2018 to 2025 by evaluating toxicological and demographic information related to gabapentin positive results in DWI casework. Case studies will be presented.

Methods: DWI case samples submitted to the Toxicology Laboratory at SWIFS between January 1, 2018, and December 31, 2025, were reviewed for positive gabapentin results in blood. The individual demographics and co-occurrence with other drugs were evaluated.

Results: Over the 8-year period, SWIFS received 23,299 DWI cases, 87 of which were positive for gabapentin (0.4%). Positive cases per year ranged from 0 in 2018 to 19 in 2021. For DWI casework the average gabapentin concentration in blood was 3,798.1 ng/mL (median 2,343.5 ng/mL, range 201.9 – 16,008.0 ng/mL, n=36).

Users were 54% male, 29% female, and 17% Unknown. Race demographics showed users were 49% White, 33% Black, and 17% Uknown. The average age was 44 years and ranged from 20 to 79 years.

Stimulants, benzodiazepines, and THC and its metabolites were most commonly identified with gabapentin. Amphetamine was the most prevalent stimulant, and alprazolam was the most prevalent benzodiazepine.

Discussion: Gabapentin has gained a growing interest in the field of forensic toxicology as a result of its increasing prevalence due to the wide range of prescription purposes, both approved and off-label use. A large data set was available to inform the community if there was an increase

in prevalence of gabapentin over an extended period of time. However, in Dallas County, gabapentin positivity in DWI casework has remained low from 2018 to 2025. Blood concentrations were within the therapeutic range of 2 – 20 mg/L, suggesting individuals are taking gabapentin at therapeutic doses. While considered generally safe with minimal adverse effects, laboratories should consider adding it to their scope as there is precedent for impairing effects.

Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

O-042

Prevalence of Psychoactive Drugs in Injured Drivers Over 10 Years in Victoria, Australia

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Abstract

Introduction: Drug use is a major contributor to road trauma. In Australia, illicit drug use remains common (about 18% annually in 2022/23), with cannabis and cocaine most used, alongside opioids/pain relievers, MDMA and methamphetamine. Victorian hospital data from injured drivers show frequent drug detections, including methamphetamine and THC; overall, impairing substances were found in about 38% of drivers. These drugs can affect driving through impaired attention, reaction time and judgement, increasing crash risk and severity. Victoria mandates blood testing of injured drivers for THC, methamphetamine and MDMA, but other impairing substances including benzodiazepines, sedatives, opioids and emerging new psychoactive substances may also contribute to road trauma yet are not part of routine per se enforcement.

Objectives: This 10-year study involves the analysis and interpretation of ~10,000 hospital blood specimens from injured drivers (from 2014- 2023) to track changing drug patterns and inform strategies to reduce drug-related road trauma.

Methods: Blood was collected in preservative tubes (1% fluoro-oxalate) by a medical practitioner or registered nurse as soon as practicable after admission and was submitted to the Victorian Institute of Forensic Medicine for routine analysis. To better characterize drug involvement beyond the mandated panel, Victoria Police project officers then used Microsoft Excel–based formulae to randomly select approximately 83 cases per month for expanded, comprehensive toxicology. This produced a stratified sample of 1000 injured drivers from each of nine 12-month periods, covering July 2014 through June 2023 (total ~10000 cases), enabling assessment of temporal trends in drug detection among hospitalized crash-involved drivers.

Blood samples were analysed at the Victorian Institute of Forensic Medicine using validated LC-MS/MS methods. A semi-quantitative screen targeted up to 327 substances across major drug classes (prescription and illicit), including amphetamines, benzodiazepines, cannabinoids, cocaine metabolites, opioids/analgesics and psychotropics (and alcohol). The panel expanded over time to include additional therapeutics and emerging novel psychoactive substances, including >80 synthetic opioids (e.g., fentanyl analogues) and other NPS. A separate LC-MS/MS NPS screening method was also used and regularly updated.

Results and Discussion: Across a decade of Victorian hospitalised crash-involved drivers, potentially impairing drugs were frequently detected. Blood was collected a median 2.2 h after the crash and analysed using progressively improved, fully validated chromatographic screening that expanded the range of detectable medicines, illicit drugs and novel psychoactive substances (NPS). Overall, 38% of drivers were positive to one or more potentially impairing drugs and 14.8% had more than one impairing substance; 40% were negative to both drugs and ethanol. THC

(11.5%) and methamphetamine (12.8%) were the most prevalent illicit drugs, with THC slightly increasing in the second 5-year period and methamphetamine largely stable (but higher in motorcyclists). Benzodiazepines were common (~12% in the last 5 years) and antidepressants averaged 14.2%. The most notable trend was the rise of NPS from first detections in 2016/17 to 47 detections in 2022/23, dominated by novel benzodiazepines and usually co-occurring with other drugs. Limitations include sampling delay, incomplete annual sampling and possible post-crash medication effects.

Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

O-043

Strengthening Toxicological Preparedness Through the EUDA Network of Forensic and Toxicological Laboratories

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Abstract

Introduction: The European drug market is continuing to diversify, presenting an increasing number of analytical and toxicological challenges. These challenges stem from rapid chemical innovation, limited reference data and difficulties of detecting substances with short detection windows, rapid distribution or volatility. In this context, coordinated laboratory collaboration is essential to strengthen Europe-wide toxicological preparedness and response capacity.

Objectives: This presentation outlines the recent activities of the EUDA Network of forensic and toxicological laboratories emphasising its contributions to enhancing analytical capabilities, standardising methodologies, and facilitating toxicological analysis. Important EUDA initiatives to support the work of the network, such as MASSIVE (Metabolite Analysis System Supporting In-depth Validation and Exploration) project, the distribution of reference standard kits containing new synthetic opioids, and targeted technical exchanges that address analytical challenges in biological matrices are described.

Methods: Activities of the EUDA Network of labs to date include the organisation of technical collaborative exchanges, the distribution of reference standard kits to more than 60 laboratories of the network and the launch of a GC-MS analytical library focused on newly detected new psychoactive substances (NPS). Feedback on the reference standard kits was subsequently collected through a survey circulated to all recipient laboratories. In addition, preparatory work related to the MASSIVE project has been initiated, with the planned scope and objectives to address gaps in metabolite reference data supporting toxicological detection and interpretation in biological matrices.

Results: The EUDA Network of labs has enhanced analytical capabilities across Europe by working together, holding regular online technical meetings and sharing reference materials. These online meetings have provided a dedicated forum for addressing analytical challenges in biological samples, particularly those involving substances with short detection windows, rapid distribution or volatility, such as ketamine, GHB/GBL and nitrous oxide. These discussions have supported the exchange of practical experience regarding sampling strategies, analytical sensitivity and interpretative limitations.

Survey feedback on reference standard kits distribution showed that 44% of the recipients used the kits to identify a substance for the first time, including 16% for the first time in their country, and 33% to confirm substances. Of the forensic and/or clinical toxicology laboratories who responded to the feedback survey two laboratories reported successfully identifying new synthetic opioids in real casework samples using the supplied reference standards, while others highlighted their use in developing analytical methods and including compounds in GC-MS spectral libraries.

In parallel, the MASSIVE project aims to address key limitations in metabolite reference data by supporting the identification of phase I and phase II metabolites for selected NPS. Outputs from this

project are expected to facilitate earlier detection in biological matrices and improve confidence in toxicological interpretations, particularly in acute intoxication and outbreak scenarios.

Discussion: The activities of the EUDA Network labs underline the importance of coordinated laboratory cooperation in addressing current and emerging analytical challenges. The combination of targeted online technical meetings, the structured distribution of reference standards and metabolite-focused research under MASSIVE contributes to the harmonisation of toxicological practices across laboratories, making them more sensitive and reliable. Together, these efforts support and strengthen Europe's ability to detect, interpret and respond to evolving drug-related threats.

Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

O-044

Investigating the Expanding World of Commercially Available Psychotropics

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Abstract

Introduction: The passage of the 2018 Agriculture Improvement Act ("Farm Bill") opened the door to an expanding marketplace of alkaloids and novel psychoactive substances (NPS) being sold in smoke shops. Semi-synthetic cannabinoids (e.g., delta-8-THC, HHC) were the first class of "legal highs" sold in this new regulatory era. Consumer demand for increasingly potent products led to THCP sales. Products derived from mitragynine (MG), the most abundant alkaloid in the kratom plant, have followed a similar trajectory. 7-Hydroxymitragynine (7OHMG) is present in kratom at low concentrations but is also an active metabolite of MG and is more potent. Since the U.S. Drug Enforcement Administration withdrew their intent to schedule MG and 7OHMG, manufacturers have increasingly sold commercial preparations of high-dose MG and 7OHMG products. More recently, the market has seen the emergence of more potent alkaloids like mitragynine pseudoindoxyl (MP) and dihydro-7-OH mitragynine (aka MGM-15). In more recent years, there has been an increase in "mushroom extract" products being sold that contain psilocin and/or chemical derivatives (e.g., 4-AcO-DMT) that promise psychedelic and nootropic effects. This emerging landscape contains many drugs with effects are not well understood and may pose risks to consumers.

Objectives: This presentation describes products sold at retail smoke shops in the United States with an emphasis on the identification of NPS. The packaging of retail products is misleading, often intentionally so. There will be a comparison between advertised and identified substances. Testing of these products is crucial for public health authorities who may be responding to adverse reactions resulting from unwitting customers consuming potentially dangerous products.

Methods: Commercial products were purchased from a variety of smoke shops across Maryland, Washington, D.C., Delaware, and Pennsylvania. All products were qualitatively analyzed by GC-MS and LC-QTOF-MS using non-targeted acquisition methods in comparison to an in-house library containing over 1,200 compounds. Additionally, samples containing kratom-related alkaloids (e.g., MG, 7-OHMG, MP) were quantitatively analyzed by LC-QTOF-MS using a validated assay.

Results: Of the 82 products purchased, 40 were advertised as containing a kratom alkaloid, 20 were advertised or implied to contain a psychedelic "mushroom extract", 11 were advertised as cannabinoid products, and 11 were advertised with other psychotropic substances. Most kratom products tested were labeled as containing 7-OHMG and/or MP. Quantitative analysis revealed that the amount present could be different than what was on the packaging. Most of the "mushroom extract" products contained at least one unscheduled, substituted tryptamine but several contained psilocybin and/or psilocin as well. Miscellaneous products were labeled as containing Cat's Claw extract, "mad honey", or a variety of other psychotropic compounds, none of which were detected through testing.

Discussion: The results of this ongoing study demonstrate the need for surveillance testing of products sold in self-regulating markets. Consumers are unable to obtain accurate information regarding the substances present and instead rely on online user reports. The lack of transparency involving smoke shop products is of great concern to the forensic toxicology community as laboratories must be aware of novel substances that are contributing to adverse events and overdoses.

Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

O-045

Integrated Toxico-Surveillance and Early Warning System in New South Wales, Australia: Government-Led Model With Multiagency and Community Partnerships

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Abstract

Introduction: An effective early warning system requires triangulation of multiple data sources and robust response mechanisms to communicate key information to the community.

Objectives: To describe New South Wales (NSW), Australia toxico-surveillance and early warning system, drug and toxicological results and public health responses.

Methods: We reviewed operational records, reports, analytically confirmed drug and toxicology results, and public health responses of NSW toxico-surveillance and early warning system, centrally coordinated by the NSW Ministry of Health.

Results: Besides alcohol and tobacco, which account for most drug-related harm in NSW, conventional illicit substances were the most detected across data sources. The Prescription, Recreational and Illicit Substance Evaluation (PRISE) program provides toxicological analysis of biological samples from patients presenting with severe and unusual toxicities to NSW Health services. From July 2018 to April 2026, among 1,236 cases tested, there were 461 methamphetamine (37%), 322 cocaine (26.1%), and 304 delta-9-THC (24.6%) detections, with a median of seven drugs detected per case.

Of over 40,000 coronial cases that underwent toxicological analysis between July 2010 and March 2026, the most common illicit substances detected were delta-9 THC (19%), methamphetamine (11%), and heroin (4.6%). Out of more than 240,000 police seized samples analysed from July 2009 to December 2025, cannabis plant matter was detected in 19%, methamphetamine in 19%, and cocaine in 7.5%. In NSW Drug Checking trial at 12 music festivals from March 2025 to February 2026, 1,480 samples were tested. The common detections were MDMA (65%), ketamine (20%) and cocaine (8%).

New psychoactive substances (NPS) were detected at a very low percentage across all datasets. Some NPS detections included nitazenes (protonitazene, protonitazepyne, isotonitazene, etodesnitazene, metonitazene); other synthetic opioids (acetylfentanyl, fluorofuranylfentanyl, carfentanyl); synthetic cathinones/phenetylamines (4-fluoroamphetamine, methylone, paramethoxymethamphetamine, 2-CB, dipentylone, 25C-NBOMe); ketamine

analogues (fluorexetamine/2-fluoro-2-oxo PCE, 2-fluorodeschloroketamine); novel benzodiazepines (bromazolam, etizolam, bromonordiazepam, clonazolam), and others (xylazine, tiletamine, 1,4-butanediol). Following concerning detections, 55 public drug warnings and 20 clinical safety notices were issued from July 2018 to April 2026.

Discussion: Triangulation of multiple complementary data sources in NSW enables a comprehensive picture of drugs in circulation and causing harm. The PRISE's case identification includes statewide syndromic and drug toxicity surveillance of unplanned presentations to NSW emergency departments, and notifications from multiple sources, including, but not limited to, toxicology services, poisons information centre, clinicians and peer-based organisations. Early toxicology results support clinical management and public health interventions.

The Combined Surveillance and Monitoring of Seized Samples (CoSMoSS) program enables timely drug-testing results for samples seized by the NSW Police Force (including at music festivals).

Rapid information on the chemical content and purity of samples submitted during the NSW Drug Checking trial enabled individualised harm-reduction advice for service users. Two on-the-spot drug warnings were issued at music festivals. NSW approaches to assessing and advising the most effective means to prevent drug-related harm integrate the perspectives of consumers and a range of clinical and public health professions. Strong partnerships across government agencies and communities, political and senior leadership support, integration with the health system, and a multidisciplinary network enable the successful operation.

Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

O-046

Lessons From the UNODC Early Warning Advisory on the Changing Face of NPS and How Systems Need to Adapt

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Abstract

Introduction: Over the past decade, the global drug landscape has been reshaped by the rapid emergence of new psychoactive substances (NPS), characterised by chemical diversity, market volatility, and uneven geographic distribution. Traditional forensic and toxicological systems, historically optimised for a relatively stable set of internationally controlled substances, have been challenged by the speed, scale, and transience of these compounds. In response, the United Nations Office on Drugs and Crime (UNODC) established the Early Warning Advisory (EWA) on NPS in 2013 to support timely detection, trend analysis, and international information exchange. The UNODC EWA functions as an adaptive information-sharing system, providing real-time access to global detections across multiple data sources and matrices.

Objectives: To examine long-term trends in NPS emergence, geographic reporting and substance class dynamics, with a focus on implications for the forensic community.

Methods: Descriptive and trend analyses were conducted using aggregated EWA surveillance data from 2008 to April 2026 (2013-24 are comparator years due to data completeness). Indicators examined included numbers of individual NPS reported over time; numbers of reporting countries or territories; first-time reports of substances; distribution by structural and drug effect group; and regional reporting patterns.

Results: EWA grew from monitoring 502 unique NPS reported in drug samples across 94 countries in 2013 to 1,465 from 154 countries and territories. Annual reporting volumes showed marked variability, ranging from 449 substances in 2013 to a peak of 755 in 2024. First-detections of NPS demonstrated sustained innovation, ranging from 47-136, with notable peaks in 2015, 2021, and 2024.

When examining the diversity of NPS, synthetic cannabinoids remained the most common class (35% in 2013 to 27% in 2024). Stimulants saw a slight shift from phenethylamines (19% in 2013 to 12% in 2024) to synthetic cathinones (14% in 2013 to 16% in 2024). Synthetic opioids saw marked changes from 1% of NPS in 2013 to 11% in 2024 – within this group, discrete waves of fentanyl analogues, followed by nitazenes and most recently orphine analogues were observed. Benzodiazepines also saw marked changes from 1% in 2013 to 5% in 2024. Post-mortem and clinical toxicology samples, as key indicators of harm, show that benzodiazepines are the most commonly detected NPS (37%), and this correlates with the frequency of drug sample detections.

Regionally, there were notable increases in reporting of synthetic opioids initially in North America, Europe and Asia, followed by dominance in North America but in more recent years being more widespread and the emergence in Oceania, South America, and Africa.

Discussion: These findings demonstrate that NPS continue to emerge over time, with no indication of slowing, but with changes observed in both structural groups and geographic distribution – presenting new challenges for forensic and toxicology laboratories. Persistent

limitations include incomplete toxicological characterisation for many newly detected substances and increasing complexity of interpreting polysubstance exposure. Forensic professionals contribute centrally to this process by interpreting analytical data to inform early-warning outputs. Experience shows that effective early warning for NPS relies not only on analytical capability, but also on contextual interpretation, cooperation between countries, and toxicology-informed prioritisation to support appropriate responses in an increasingly dynamic drug landscape.

Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

O-047

The Potential Displacement of 'Nitazenes' with 'Orphines' on the Novel Synthetic Opioid Market

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Abstract

Introduction: The novel psychoactive substance (NPS) landscape can rapidly shift from one substance/subclass to another in response to control efforts. In July 2025, the Chinese government placed 'nitazene' analogues under generic control. Although *N*-propionitrile chlorphine (cyclorphine), a chemically distinct novel synthetic opioid (NSO), was first reported in the United States (US) in December 2024, it quickly started to proliferate late 2025, while positivity of nitazene opioids declined. *N*-Propionitrile chlorphine is succeeding brorphine, which first emerged mid-2020 but subsequently declined after domestic scheduling, as the second most popular NSO of the benzimidazolone or 'orphine' subclass. The 'orphine' subclass continues to rapidly proliferate and diversify, with additional compounds chlorphine (a *N*-propionitrile chlorphine metabolite), spirochlorphine, and 3,4-dichloro desmethylchlorphine also detected in casework. NSOs are often found at low concentrations due to their elevated potency, and diverse mixtures of nitazenes and/or orphines continue to challenge laboratories who need to rapidly expand testing capabilities to stay current with dynamic drug markets.

Objectives: This study aims to characterize the evolving temporal, geographic, and toxicological trends specific to nitazenes and orphines to inform laboratory testing strategies.

Methods: Toxicology data from 2025 through Q1 2026 were reviewed for reporting of nitazenes and orphines. Twenty-three compounds of these subclasses, including metabolites, are included in the testing surveillance library for datamining of high-resolution mass spectrometry-based data files. Cases in which confirmatory testing was performed for nitazenes and orphines were coordinated between NMS Labs and the Center for Forensic Science Research and Education (CFSRE).

Results: Our data illustrates the corresponding shift in positivity to orphines following control efforts for nitazenes. There were 176 confirmed blood cases reporting one or more nitazene compounds (isotonitazene, protonitazene, metonitazene, *N*-pyrrolidino etonitazene, *N*-desethyl isotonitazene and *N*-pyrrolidino protonitazene) in 2025, which decreased 49% from 350 nitazene-involved cases in 2024. There were 69 cases confirmed positive for nitazene(s) in Q1 2025 prior to the Chinese scheduling; comparatively, there were eleven cases reported in Q1 2026. Another 17 cases of the less prevalent nitazenes were reported by the CFSRE in 2026. Reported compounds included *N*-pyrrolidino isotonitazene, *N*-pyrrolidino metonitazene, etonitazene, and *N*-pyrrolidino ethylene isotonitazene. In late 2025, 15 blood samples forwarded to the CFSRE confirmed positive for *N*-propionitrile chlorphine, with an additional 46 cases reported in the first quarter of 2026.

Geographical trends are difficult to assess for emerging substances due to patchwork surveillance and variable testing practices. Currently, *N*-propionitrile chlorphine has been identified in samples submitted from 12 US states, Canada, and the United Kingdom.

Discussion: The orphine subclass is poised to replace the nitazene subclass on the NSO market. However, mixtures of nitazenes and orphines have been reported in several cases, in what appears to be regionalized outbreaks (e.g., Midwest). This diverse mixture of potent NSOs poses significant health risks to end users, in addition to significant analytical and interpretative challenges to toxicologists. The NPS market continues to shift in response to control efforts with displacement and diversification rather than eradication; laboratories must be prepared to quickly update their scopes in response. *N*-Propionitrile chlorphine serves as the latest NSO *du jour*.

Disclosure

Yes, I, or a member of my immediate family, has a financial interest to disclose.

Conflict of Interest

Salaried employee of NMS Labs

O-048

Recent Decline of Designer Opioid Positivity in Clinical Specimens

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Abstract

Introduction: Since 2019, laboratories have seen an increase in the number of non-fentanyl designer opioid positive specimens. Once the most prevalent compounds in the designer opioid class, nitazene analogs are now slowly being overtaken by analytes such as brorphine and propionitrile chlorphine (cychlorphine), collectively referred to as the "orphines". This is likely due to the increased regulation of nitazenes in recent years. Most notably, China introduced a class-wide scheduling of nitazenes in June 2025, leading to a decrease in non-fentanyl opioid positivity.

Objectives: This study reviews the overall declining trend of designer opioids (DOs) over an 18-month period, from July 2024 to December 2025, focusing on the nitazene analog decrease and introduction of orphine compounds.

Methods: This study retrospectively evaluated clinical urine specimens submitted to Quest Diagnostics for novel psychoactive substances (NPS) testing between July 2024 and December 2025. An annually updated semi-quantitative LC-MS/MS method was used for analysis. NPS in the method were separated into 6 classes, one of them being the DO class, which includes 22 analytes spanning both parent and metabolite nitazene and orphine analogs. Cutoff concentrations in the DO class ranged from 1-10 ng/mL. Data was analyzed to discern trends in DO positivity during the investigated time period.

Results: While specimens submitted for NPS testing originated from all over the United States, the majority of DO positive specimens came from the Midwest region. From July to December 2024, there was a general increase in DO detections in positive specimens from 4.9% to 6.0%. Pyrrolidino hydroxy-nitazene (n=203) and hydroxy-nitazene (n=105) were the two most detected metabolites, while metonitazene was the most common parent analyte (n=52). Brorphine was identified 7 times and was the only orphine included in the test panel during this time.

Throughout 2025, desethyl metonitazene (n=229), pyrrolidino hydroxy-nitazene (n=193), and metonitazene (n=119) were the 3 most identified analytes, while brorphine was identified just once. Dichloro desmethylchlorphine and propionitrile chlorphine (cychlorphine) were identified 3 and 5 times, respectively, after their inclusion into the panel in October 2025 during a generational update.

The first decrease in DO positivity was observed between December 2024 and January 2025. DO class positive specimens accounted for 3.9% of all positives in January 2025, a 35% decrease from December 2024 (6.0%). By the end of 2025, there was a sharp decrease in analyte positivity, specifically from October to November. In October 2025, DO positive specimens accounted for 2.4% of all positives while November positivity decreased to just 1.3%. In total, the number of positive DO specimens decreased 78% from December 2024 to December 2025.

Discussion: Over the course of 2025, DO positivity markedly decreased. Specifically, reduced nitazene positivity drove this decline, likely due to individual and class-wide scheduling. However, this trend was still apparent even with the addition of new orphine analogs at the end of 2025. Laboratories need to be aware of these changes as new compounds can quickly emerge or disappear from the always evolving NPS market.

Disclosure

Yes, I, or a member of my immediate family, has a financial interest to disclose.

Conflict of Interest

Salary

O-049

From 4-MMC to 2-MMC: Wastewater-Based Evidence of an Isomeric Shift in Methylmethcathinone Use in Belgium

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Abstract

Introduction: Synthetic cathinones are the second largest group of new psychoactive substances (NPS) monitored by the European Union Drugs Agency, with increasing methylmethcathinone (MMC) seizures and self-reported consumption in surveys. However, assessing community-level consumption remains challenging due to the rapidly evolving NPS market, varying legislation, and frequent unintentional use of mislabelled substances. In addition, chromatographic separation of the three MMC isomers (i.e., 2-, 3-, and 4-MMC) remains analytically challenging, yet is essential given their distinct pharmacological profiles and regulatory status.

Objectives: This study applied wastewater-based epidemiology (WBE) in Belgium to investigate spatio-temporal trends in MMC consumption.

Methods: An analytical workflow based on solid-phase extraction and liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS) was developed and validated for the separation and quantification of 2-, 3- and 4-MMC in influent wastewater (IWW). Chromatographic separation was performed on a Phenomenex Kinetex Biphenyl column (2.1 mm x 100 mm, 2.6 µm particle size). The analytical quantification range was set from 4 to 500 ng/L. Daily 24-h composite IWW samples were collected from ten locations across northern Belgium (Flanders and the Brussels region) in 2023 and 2025 (n = 311). Additional IWW samples collected between 2019–2022 and in 2024 were analysed for Brussels and Antwerp. Measured concentrations in IWW were transformed to population-normalised mass loads (PNML, expressed in mg/day/1000 inhabitants), accounting for wastewater flow and the population served by the wastewater treatment plant catchment area, serving as a proxy for MMC consumption.

Results: MMC use is widespread across Flanders, occurring in both major urban centres and smaller municipalities. Increasing consumption trends were observed over the study period. Furthermore, an isomeric shift in MMC consumption was observed over time. Although 4-MMC was historically the predominant MMC isomer following its emergence around 2010, only minor levels were detected in this study. In contrast, 3-MMC was the dominant isomer in 2023, whereas by 2025 its consumption had declined substantially alongside a marked increase in 2-MMC in all monitored locations. This could be linked to legislative changes the Netherlands, but more research is required.

Discussion: This study illustrates the strengths of wastewater-based epidemiology to provide near real-time, objective measurements of illicit drug consumption. A rapidly evolving MMC market in Belgium is revealed, with increasing consumption and a clear isomeric shift from 3-MMC to 2-MMC. These findings highlight the importance of monitoring all MMC isomers to draw correct conclusions on MMC consumption patterns, which can be used to support evidence-based drug policy (re-)evaluation. Furthermore, this study highlights the value of WBE as a complementary data source in forensic toxicology, supporting laboratories in prioritizing target compounds—including isomers—and associated trends, thereby helping to ensure that relevant emerging substances are not overlooked in casework.

Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

O-050

Wastewater-Based Epidemiology as a Complementary Tool to Multimodal New Psychoactive Substance Toxicosurveillance

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Abstract

Introduction: New psychoactive substances (NPS) pose ongoing challenges for public health due to their rapid emergence and rising harms. Although there are different surveillance systems to monitor their emergence and prevalence including surveys, seized material, clinical toxicology data, forensic reports, and wastewater, their integration remain fragmented.

Objectives: To examine international surveillance of NPS by examining three indicator sources: detection in wastewater compared with toxicology and drug seizure data reported to the United Nations Office on Drugs and Crime Early Warning Advisory (UNODC EWA). This approach enables concurrent assessment of population-level exposure (wastewater), supply dynamics (seized material and collected samples such as from drug checking services), and clinical harm (toxicology), and explores methodological gaps in current NPS surveillance.

Methods: In this retrospective secondary data analysis, wastewater-based epidemiology data were collected from studies published over 2014-25 and data reported to UNODC EWA, covering sampling over 2012-25. Drug and toxicology sample reports were obtained from the UNODC EWA, covering sampling over 2012-25. Noting, NPS analytical and reporting practices vary significantly between countries, particularly for wastewater. Data were harmonised by extracting and aligning compounds, countries and years by data source. Interactive data visualisations were produced and analysed for key trends from a data triangulation perspective.

Results: A total of 143 NPS were reported in wastewater studies from 35 countries and 1468 NPS from 147 countries to UNODC EWA. One example of an NPS detected across all three indicator sources is mitragynine. It has been identified through wastewater analysis in 14 countries since 2019, while reports to EWA have been submitted from 57 countries since 2012. Wastewater was the last matrix to report mitragynine in all but one country, with Czechia being the sole exception where wastewater detection occurred concurrently with drug seizures. In the example of Australia, mitragynine was first reported in 2018-20 in toxicology samples only, then 2021-2 in wastewater only, followed by 2023-4 in all 3 indicator sources and for 2025 in wastewater and toxicology samples. MDPEP provides an example where the first detection in Australia was in wastewater in 2022-3, followed by drug samples in 2024. The example of bromazolam highlights the first detection in drug samples in 2016, followed by toxicology samples in 2021, then wastewater in 2022. In 2022, 29 countries reported bromazolam in drug samples, 8 in toxicology samples and 5 in wastewater samples. The substantial discrepancy in detection across countries and indicator sources highlights key knowledge gaps, particularly countries where wastewater analysis could complement existing surveillance data.

Discussion: This study demonstrates the value of integrating different data sources for complementary surveillance of NPS. While one key limitation of the UNODC EWA is its voluntary submissions, the three indicator sources investigated in this work can be considered complementary and mutually reinforcing sources for triangulating NPS surveillance signals. Further improvement in data collection and exchange of multiple complementary indicator sources within and between countries will strengthen early warning responses and is advocated in UNODC resolutions. The UNODC EWA data platform is a key enabler to achieve this goal.

Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

O-051

From Signal to Surveillance: A Wastewater-Based Forensic Intelligence Framework for Emerging Drug Threats in Türkiye

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Abstract

Introduction: Wastewater-based epidemiology (WBE) provides population-level, near-real-time drug consumption data complementary to law enforcement reporting. In Türkiye, a nationwide WBE mission operational since 2019 has established a validated surveillance infrastructure. Between 2022-2024, Turkish law enforcement data reveal a 6.4-fold increase in pregabalin seizures, alongside a six-fold rise in tramadol seizures. Ketamine is increasingly flagged by EUDA/UNODC as an emerging threat, yet no domestic seizure data exist. Penetration of new synthetic opioids (NSOs) across Europe, driven by fentanyl analogues and nitazenes, is relevant given Türkiye's position on the Balkan route. These converging signals demanded expansion of the existing infrastructure.

Objectives: This study presents the signal-driven expansion of Türkiye's national WBE policy to incorporate pregabalin, ketamine, tramadol, xylazine, NSOs (nitazenes and fentanyl analogues) and new benzodiazepines, positioning the existing surveillance infrastructure to detect and quantify emerging substance threats ahead of conventional law enforcement visibility.

Methods: Pregabalin and ketamine and its main metabolite norketamine, were incorporated into two previously validated LC-MS/MS methods, enabling rapid deployment. Composite samples from 31 cities (anonymized for confidentiality; ~37.5 million) were analyzed for pregabalin, ketamine and norketamine across different periods. Population-normalized mass loads (PNMLs; mg/day/1,000 inhabitants) were calculated from concentrations, daily flows, and population. To extend coverage to tramadol, xylazine, NSOs, and new benzodiazepines, a dedicated LC-MS/MS method (supported by TUBITAK 124Z874) incorporating optimized SPE procedure is currently under development and validation, which will be applied to 2026 monitoring.

Results: Pregabalin was detected in virtually all sampled cities across all periods. Cross-national mean PNMLs were broadly stable across 2023–2024 (646 and 586 mg/day/1,000 inhabitants), a pattern not directly mirroring the sharp seizure escalation over the same interval, suggesting partially distinct dynamics. The near-doubling of mean PNMLs in 2025 to 1,588 mg/day/1,000 inhabitants — the highest recorded — provides the only available evidence that this trajectory has continued to accelerate beyond current law enforcement reporting. Ketamine was detected in 30/31 cities across the surveillance window, providing nationwide consumption estimates across 2024–2025. Median PNMLs of 0.20–0.48 mg/day/1,000 inhabitants reflected low but geographically widespread consumption. The elevated cross-national mean observed in 2024 was driven by two cities with disproportionately high ketamine PNMLs where norketamine levels were only moderately elevated, raising the possibility of non-consumption sources. Norketamine was co-detected in an average of six cities per period, with mean PNML 0.25 mg/day/1,000 inhabitants and no meaningful change across 2024–2025, consistent with active use. Additionally, the first wastewater-based data on tramadol, xylazine, NSOs, and new benzodiazepines in Türkiye will be reported.

Discussion: This study demonstrates that an established WBE policy can be rapidly expanded to serve as a proactive forensic intelligence tool, incorporating analytes in direct response to international threat signals before domestic law enforcement visibility. The ketamine and pregabalin findings, together with forthcoming data on NSOs and other emerging substances, exemplify this capacity and suggest that routine forensic and clinical toxicology panels may warrant similar expansion to close corresponding detection gaps. Collectively, these findings support the integration of WBE into national drug early-warning architectures as a complementary forensic intelligence layer.

Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

O-052

Wastewater-Based Epidemiology as an Early Indicator and Preparedness Tool: Rapid Changes in Synthetic Cathinone Markets in Finland

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Abstract

Introduction: Synthetic cathinones (SCs) are increasingly available in Europe, where they represent the largest proportion of seized quantities and are increasingly replacing traditional stimulants such as amphetamine. The increase in cathinone consumption highlights the importance of forensic toxicology laboratories and innovative monitoring tools for understanding the scale and adverse effects of the phenomenon. Wastewater-based epidemiology (WBE) may provide early-warning information and strengthen preparedness, supporting evidence-based public health and law enforcement responses.

Objectives: To evaluate wastewater surveillance as an early indicator and objective metric of SC consumption based on a large-scale national monitoring program in Finland. A secondary objective was to compare temporal and spatial patterns in wastewater data with those obtained from other available drug monitoring tools.

Methods: Both methodological pillars of the national wastewater monitoring program in Finland were utilized. First, nationwide sampling campaigns were conducted at up to 32 wastewater treatment plants (WWTPs), currently covering nearly 65% of the Finnish population, between 2012 and 2026. Second, near real-time monitoring using 24-hour influent composite samples (2013–2026, n = 282) was conducted under comparable conditions at the largest WWTP in the Nordic countries (Helsinki WWTP), serving approximately 930,000 inhabitants. All samples were analysed using a validated UHPLC-MS/MS method. The method currently targets approximately 25 SCs, and samples were additionally screened using QTOF/MS with a large in-house database. The selection of relevant SCs for accurate quantification was based on qualitative wastewater monitoring, toxicological and seizure data, as well as information from user forums. Correlations between the most common SCs detected in wastewater and laboratory-confirmed data, including driving under the influence of drugs (DUID) cases, postmortem toxicology, drug seizures, and substances detected in used syringes, were evaluated.

Results: Alpha-PVP remains the predominant SC and new psychoactive substance in Finland. Based on WBE data from the capital area of Finland (Helsinki WWTP), alpha-PVP use declined following its classification under the Narcotics Act at the end of 2013, but started to increase sharply in early 2022. Mean alpha-PVP mass loads (average ± SD) increased from 0.74 ± 0.92 mg/1000 persons/day in 2021 to 3.8 ± 2.0 in 2022, 11 ± 4.1 in 2023, 22 ± 7.0 in 2024, 31 ± 7.6 in 2025, and 35 ± 2.9 in 2026 (until the end of February). The detection frequency in wastewater was 100% throughout this period. Similar trends were observed in nationwide WBE data, although with notable geographical variation: alpha-PVP use was highly concentrated in the capital region and in southern and southwestern Finland. The number of alpha-PVP–positive DUID cases showed a comparable increase: 124 cases in 2021 (1.3% of all suspected cases), 641 (7.4%) in 2023, and 951 (12%) in 2025. In addition, WBE indicated emerging signals, such as regular detections of MMCs in early 2026, while previously common

substances such as MDPV, alpha-PHP, and PiHP have largely disappeared from the Finnish drug market.

Discussion: The high sensitivity and applicability of WBE to SCs support its use as an efficient early indicator and key preparedness tool in rapidly changing drug markets. In practice, monitoring should include a broad range of SCs qualitatively, with quantitative analyses focused on the most prevalent compounds.

Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

O-053

A Tri-State Surveillance Study of Regulated and Unregulated Cannabinoid Products

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Abstract

Introduction: The rapid expansion of the cannabis industry has outpaced regulatory oversight. Semi-synthetic cannabinoids (e.g., Δ8-tetrahydrocannabinol (Δ8-THC) and hexahydrocannabinol (HHC)), synthetic cannabinoids (e.g., THC-acetates), solvents, and microbiologicals are often identified in unregulated products. Robust product surveillance is essential to protect public health and inform evidence-based regulation and enforcement as access to, and demand for, cannabis products increases.

Objectives: To identify the psychoactive compounds, solvents, and microbiologicals in cannabis products (concentrates, dog treats, drinks, edibles, plant material, tabs, tinctures, THC diamonds, and vapes) purchased in Virginia, North Carolina, and New York.

Methods: Products were purchased from regulated and unregulated cannabis, vape, tobacco and convenience stores. Product type, brand, label information, and, when available, website and certificate of analysis (CoA) details, were recorded. All samples were evaluated by previously published methods including: screening by a gas chromatography-mass spectrometry (GC-MS) method, volatile analysis by headspace gas chromatography-flame ionization detection (HS-GC-FID) and cannabinoid quantitation by liquid chromatography-tandem mass spectrometry (LC-MS/MS). Microbiological (yeast, mold, aerobic count, coliform, and E. coli) contamination was assessed using Neogen Petrifilm® plates.

Results: Analyzed products were: 30 vapes, 21 edibles, 5 beverages, 39 flower/pre-rolls, 2 concentrates, 2 tabs, 1 tincture, 1 THC diamonds, and 1 bag of dog treats. Labelling indicating ingredients and/or concentrations was found on 40%, and CoA on 65%. Both labels and CoA were found for 36 products and all 36 products had inconsistencies between label and CoA. All solvents were detected below 10 mg/mL at the following prevalence: methanol (43%), ethanol (29%), and isopropanol (1%). The following cannabinoids were detected (by prevalence): Δ9-THC, cannabinol (CBN), Δ8-THC, cannabigerol (CBG), cannabicitran (CBT), tetrahydrocannabivarin (THCV), cannabichromene (CBC), cannabidiol (CBD), Δ8-iso-THC, cannabicyclol (CBL), cannabivarin (CBDV), Δ4(8)-iso-THC, cannabidihexol (CBDH), exo-THC, Δ8-tetrahydrocannabibutol (Δ8-THCB), iso-HHC, β-hydroxy-HHC (β-OH-HHC), HHC, Δ9-tetrahydrocannabiphorol (Δ9-THCP), Δ6a-10a-THC, hexahydrocannabivarin (HHCV), Δ8-tetrahydrocannabinol acetate (Δ8-THC-O), cannabibutol (CBDB), cannabiphorol (CBDP), cannabielsoin (CBE), tetrahydrocannabinolic acid (THCA), Δ8-THCP, and hexahydrocannabiphorol (HHCP). Total cannabinoid concentrations ranged from 0.1% to 99.0%. Microbiologicals above US Pharmacopeia thresholds were found in 40% of products, and 56% of those contained two or more. Prevalence was: total yeast and mold (68%), aerobic bacteria (76%), and coliform (39%). VA products had the highest microbiological prevalence.

Discussion: Most products either contained undeclared cannabinoids, did not contain the declared cannabinoid, or were determined to be +/-20% of labelled concentration. Discrepancies were noted between product labeling and CoA (e.g., ingredients present, concentrations). Not a single state had an agreed product labelling, CoA, or determined cannabinoid content. These findings demonstrate issues with quality assurance that can also lead to consumer confusion and adverse reactions due to incorrect information. Low concentrations of volatiles were identified in 59% of the products. Microbiologicals were most often found in plant material. Every state had products with microbiological growth above the US Pharmacopeia thresholds.

These findings underscore the risks associated with unregulated cannabis products. Continued surveillance and regulatory enforcement are essential to ensure safety and product integrity in the expanding cannabis market.

Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

Conflict of Interest

National Institute of Justice [15PNIJ-23-GG-014210COAP]. The opinions, findings and conclusions expressed are those of the author(s) and do not reflect those of the Department of Justice.

O-054

From Paper to Powders: Variability in Synthetic Cannabinoid Purity in Scottish Prisons

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Abstract

Introduction: Following the introduction of mail photocopying in Scottish prisons in 2021, patterns of synthetic cannabinoid receptor agonist (SCRA) supply has shifted from impregnated paper and greeting cards towards powders and waxy materials. This change has coincided with an increase in SCRA-related incidents within the prison estate, despite little change in the types of SCRAs being detected. Alongside this shift, seizures of vape pods have increased, and vaping is suspected to be the primary route of administration for both impregnated papers and powdered synthetic cannabinoids within Scottish prisons, with powders added to vape liquid and impregnated papers placed in contact with the heating element prior to use. These changes have important implications for toxicological interpretation, particularly in relation to dose estimation and the risk of severe intoxication.

Objectives: This study developed and applied a quantitative analytical approach for MDMB-4en-PINACA, ADB-BUTINACA and MDMB-FUBINACA using gas chromatography-mass spectrometry (GC-MS) to measure the average SCRA content across different dosage-formats seized in Scottish prisons, with a focus on comparing the amount of drug present in infused papers versus powders and to assess differences in potential exposure.

Methods: Quantitative method validation followed American National Standards Institute (ANSI) and American Academy of Forensic Science Standards Board (ASB) Standard 036, assessing Limit of Detection (LOD), Limit of Quantitation (LOQ), precision, and bias.

Non-attributable seized samples were provided by the Scottish Prisons Service as part of the Scottish Prisons Non-Judicial Drug Seizure Monitoring Project. Paper samples (1 cm^2) were extracted in methanol with bupivacaine internal standard (100 µg/mL) by ultrasonication; powder samples (5 mg) were dissolved in methanol.

GC–MS analysis was performed using an Agilent 7820A gas chromatograph coupled to an Agilent 5977E mass spectrometer (Agilent Technologies, USA). Samples (1 µL) were injected (20:1 split) onto an HP-5MS capillary column (25 m × 0.2 mm × 0.33 µm) with helium (1.0 mL/min). The oven was programmed from 80 °C (3 min) to 300 °C at 40 °C/min (6 min hold; total 14.5 min). The MS operated in EI mode (70 eV, 50–550 m/z).

Results: Results demonstrate substantial variability in SCRA content between formats, with powders presenting significantly higher potential exposure to an individual when vaping, primarily due to a greater overall dose potential, although differences in vaping efficiency between matrices likely also contribute. Using MDMB-4en-PINACA as an illustrative example, impregnated paper samples contained an average of 3.9 mg per cm^2. In contrast, powder samples showed a mean concentration of 658 µg per mg. When typical amounts of powder used to fill vape pods are considered (approximately 275 mg), this corresponds to an estimated total dose of 181 mg of MDMB-4en-PINACA per pod.

Discussion: These findings demonstrate that there could be significant toxicological consequences for those unaware of the differences in the dosage units when vaping powered materials compared to infused papers. This provides a plausible explanation for the increased severity and frequency of SCRA-related adverse events observed within the Scottish prison estate. The results have direct relevance for forensic and clinical toxicology, supporting improved interpretation of casework involving SCRAs and highlighting the importance of considering drug format and unit-dose when assessing exposure risk.

Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

O-055

Chemometric-Driven Optimisation and Validation of Phosphatidylethanol Quantification in Dried Saliva Spots: A Green, Non-Invasive Approach for Forensic Alcohol Biomonitoring

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Abstract

Introduction: Alcohol consumption remains a key focus in forensic investigations, with phosphatidylethanol (PEth) in dried blood spots (DBS) recognized as a highly specific direct biomarker of ethanol intake. Since PEth is considered to be formed in cell membranes and can be detected in biological matrices containing phosphatidylcholines, alternative matrices beyond blood may also be suitable for PEth testing. Dried sampling approaches offer notable advantages, including simplified collection, improved stability, and easier storage and transport. However, conventional sampling strategies still face limitations such as invasiveness, logistical challenges, and environmental burden. These drawbacks highlight the need for minimally invasive and sustainable alternatives. In this context, dried saliva spot (DSS) sampling has emerged as a promising microsampling approach, as saliva represents a non-invasive biological matrix that may support PEth analysis while further reducing invasiveness and maintaining practical and environmental benefits.

Objectives: This study aimed to develop and fully validate a sensitive and robust liquid chromatography-tandem mass spectrometry (LC–MS/MS) method for the quantification of PEth homologues (16:0/18:1 and 16:0/18:2) in DSS as an alternative biological matrix. Furthermore, a chemometric approach was employed to systematically identify and optimize critical analytical parameters influencing extraction efficiency and detection performance.

Methods: PEth homologues were quantified using LC–MS/MS. A Plackett–Burman experimental design was employed to screen key variables affecting analytical performance, including sample volume, drying time, extraction solvent volume, evaporation temperature, shaker time, and reconstitution volume. Method validation was conducted in full accordance with ANSI/ASB Standard 036. In addition, the environmental impact of the developed method was systematically evaluated using the Analytical GREEnness (AGREE) metric. The applicability of the method was further assessed using authentic paired DSS samples (n = 14).

Results: The method demonstrated high analytical sensitivity, with limits of quantification (LOQ) of 0.2 and 0.3 ng/mL and limits of detection (LOD) values of 0.07 and 0.1 ng/mL for PEth 16:0/18:1 and PEth 16:0/18:2, respectively. Recovery values exceeded 85% at low (12.5 ng/mL) and high (200 ng/mL) QC levels and 91% at the medium QC level (100 ng/mL). Chemometric optimization identified the most influential parameters and established optimal conditions: 10 µL sample volume, 275 µL extraction solvent (10% isopropanol (IPA) in acetonitrile (ACN)), and an evaporation temperature of 37 °C. The method achieved an AGREE score of 0.69, confirming a high level of compliance with green analytical chemistry principles. Application to authentic samples revealed that six of the 14 DSS samples were positive for PEth homologues.

Concentrations ranged from 3.23 to 48.2 ng/mL for PEth 16:0/18:1 and from 9.95 to 80.4 ng/mL for PEth 16:0/18:2.

Discussion: To the best of our knowledge, this study presents the first fully validated LC–MS/MS method for PEth determination in DSS, establishing a robust and non-invasive alternative for alcohol biomonitoring. The chemometric-driven approach ensured methodological resilience and significantly streamlined the experimental workload. Furthermore, an AGREE score of 0.69 underscores exceptional environmental sustainability through minimized solvent consumption and waste. By synergizing microsampling, chemometrics, and green chemistry, this research provides a transformative framework that expands the accessibility and sustainability of alcohol biomarker testing in forensic toxicology.

Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

Conflict of Interest

Grant/research support

O-056

Is Anhydroecgonine Methyl Ester in Oral Fluid a Reliable Marker of Smoked Cocaine Use?

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Abstract

Introduction: Cocaine base ("crack"), typically consumed by smoking, has shown a recent increase in Europe [1]; however, its prevalence remains substantially lower than that of cocaine hydrochloride, which is predominantly used intranasally. According to the 2025 Spanish Drug Report [2], cocaine base use is approximately seven-fold lower than that of cocaine hydrochloride. Anhydroecgonine methyl ester (AEME), a pyrolysis product of cocaine, is widely considered as a specific biomarker of smoked cocaine use; however, this assumption may be challenged when interpreting real-world data.

In 2025, oral fluid (OF) samples collected from drivers and submitted for confirmatory analysis following roadside drug screening were reanalyzed using a broad-spectrum LC-HRMS method to expand the range of detectable compounds. Among 470 cocaine-positive samples, AEME was detected in 61.7% of cases, a finding inconsistent with the reported low prevalence of smoked cocaine use.

Objectives: To determine the prevalence of AEME in OF samples from cocaine-positive drivers and to critically evaluate its reliability as a biomarker of smoked cocaine use.

Methods: A total of 115 OF samples were analyzed using a validated LC-MS/MS method for the determination of cocaine and its metabolites, including AEME, benzoylecgonine, ecgonine methyl ester, cocaethylene and norcocaine. To exclude the potential formation of AEME as an analytical artifact, OF samples were fortified with cocaine (100-5000 ng/mL) and ecgonine methyl ester (10-500 ng/mL). Additionally, 8 seized cocaine samples were analyzed to evaluate the presence of these metabolites in the source material.

Results: AEME was detected in 82.6% (n=95) of samples, with concentrations ranging from 0.5 to 23.1 ng/mL (median: 1.5 ng/mL). The AEME/cocaine ratio (n=61) ranged from 0.0015 to 0.1191 (median: 0.0068). No AEME was detected in fortified samples, excluding artifact formation under the analytical conditions. However, AEME was detected in all seized cocaine samples except one, with AEME/cocaine ratios ranging from 0.0005 to 0.0037 (median: 0.0008), lower than those observed in OF.

Discussion: In the two available controlled studies involving smoked cocaine with OF collection [3,4], AEME concentrations ranged from 1 to 4374 ng/mL (median: 32.5 ng/mL) and decreased rapidly, remaining detectable after 2 h in only 1 of 13 participants (3 ng/mL). Reported AEME/cocaine ratios in those smoked cocaine users (0.0046-0.1002; median: 0.0203) were higher than those observed in our study. In these controlled studies AEME/cocaine ratios were ≥0.01 in 75.9% of the samples, and ≥0.005 in 96.3%, whereas in our samples these thresholds were reached in 61% and 60% of cases, respectively. These data, added to the high prevalence of AEME detection in our OF samples and its presence in seized cocaine samples, suggest that AEME may partly

originate from the consumed material rather than exclusively from pyrolysis during smoking. These findings highlight the need for caution when interpreting AEME as a specific biomarker of smoked cocaine use in real-world settings.

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Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

O-057

Three-Way Comparison of Oral Fluid Transfer Methods: Evaluation of the Restek Oral Fluid Accessory, Sarstedt Salivette®, and Quantisal Alabama Department of Forensic Sciences Plunger Methodology

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Abstract

Introduction: In 2018, the Alabama Department of Forensic Sciences (ADFS) implemented a statewide oral fluid (OF) drug testing program to support DUI investigations through both roadside screening and laboratory confirmation. Oral fluid is collected for toxicology testing at ADFS using Quantisal collection devices. These devices utilize an applicator to collect OF, which is then placed into a buffer to preserve the sample. At ADFS, current practice dictates that a Quantisal accessory (plunger) is used to compress the applicator and transfer the sample-buffer mixture into a secondary container. The expected volume recovered from Quantisal tubes is approximately 1 mL OF + 3 mL buffer (4 mL total). Incomplete recovery of specimen from the applicator may limit the specimen volume available for analysis. Restek and Sarstedt have developed two devices, the Restek Oral Fluid Accessory (ROFA) and Salivette®, which enable recovery of oral fluid specimen from the applicator via centrifugation.

Objectives: This study compares three methods for transferring OF samples: Quantisal plunger, ROFA, and Salivette®. Evaluation parameters include specimen volume recovery, ease of use, and suitability for implementation at ADFS.

Methods: Thirty Quantisal samples were evaluated (n = 10 per transfer method: plunger, ROFA, and Salivette®). Each sample was weighed before and after transfer to determine specimen volume recovery (g). For the ROFA and Salivette® methods, samples were centrifuged at 4,000 rpm for 5 minutes. Unrecovered specimen was estimated by gravimetric difference, calculated as (post-transfer applicator mass – dry applicator mass). Residual specimen associated with the transfer device was quantified separately, where applicable, to partition loss between the device and the cotton applicator.

Results: In 2025, the average amount of OF transferred in ADFS casework was 2.33 mL. Average specimen volume recovery was highest using ROFA (3.88 g; 3.54 mL), followed by Salivette® (3.63 g; 3.46 mL), and the Quantisal plunger (3.48 g; 3.31 mL). This represents a 10.8% increase in specimen volume recovery for ROFA compared to the plunger method and a 6.6% increase compared to Salivette®.

The average unrecovered specimen was lowest with ROFA (0.09 g), followed by Salivette® (0.14 g) and the plunger method (0.56 g). For the plunger method, approximately 0.09 g of specimen remained within the plunger device, with an additional 0.47 g retained in the applicator.

Discussion: The results demonstrate trade-offs among the three transfer methods. ROFA provided the greatest specimen volume recovery, followed by Salivette® and the plunger method. However, ROFA and Salivette® required additional processing and introduced operational logistics. The plunger method resulted in the lowest specimen volume recovery but was the simplest and most operationally efficient.

Differences in specimen volume recovery may impact analytical sensitivity, particularly in low-concentration cases with limited sample volume. While higher-recovery methods improve yield, they may reduce throughput in high-volume laboratories. Method selection should balance specimen volume recovery with operational efficiency.

Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

O-058

Development of an *in vitro* Model as a Basis for Characterizing Drug Accumulation in Dental Biofilms

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Abstract

Introduction: Dental biofilms (plaque) have been identified as promising alternative matrix for detecting drugs of abuse consumption. Previous studies have shown interindividual differences in drug concentrations in oral biofilms. This suggests that individual factors like the oral microbiome or the localization of biofilm in the mouth influence drug accumulation in oral biofilm. Research into these factors is necessary for the utilisation of dental biofilms in forensic toxicology.

Objectives: The study aims to develop an in-vitro model to investigate the interindividual factors that lead to (local) differences in drug accumulation within the dental biofilm. Therefore, a reproducible and efficient in-vitro cultivation method for biofilm-forming oral microorganisms has to be developed and optimised. Furthermore, it was necessary to develop a method to quantify a higher number of drugs of abuse than in previous studies in low to sub milligram quantities quantities of oral biofilm.

Methods: A biofilm reactor with four parallel flow-through chambers was used to simulate physiological conditions in the oral cavity (DFR 110-6AL from Biosurface). The reactor was equipped with a dynamic nutrient supply (Tryptic Soy Broth, TSB), temperature control and has moderate levels of shear forces. Each monospecies of *Staphylococcus oralis*, *Actinomyces naeslundii*, *Enterococcus faecalis*, *Streptococcus sanguinis* and *Streptococcus mutans* was cultivated over four days to form biofilms. Additionally, multispecies biofilms were cultivated for up to 14 days and different chamber layouts were examined. Lyophilized biofilm served as the blank matrix for validating the LC-MS/MS method. The material was homogenized using a ball mill and extracted with acetonitrile. A LC-MS/MS method was developed for quantitative analysis. Various columns, mobile phase compositions and ionization and fragmentation parameters were investigated. The method was validated according to the guidelines of the German Society of Toxicological and Forensic Chemistry (GTFCh).

Results: NA reproducible biofilm growth was achieved within different periods of time. The dynamic flow pattern of TSB consists of a flow rate of 6 ml/min for 30 seconds, followed by a 300-second break. The LC-MS/MS method for the analytes amphetamine, methamphetamine, cocaine, benzoylecgonin, ecgoninmethylester, ketamine, norketamine, opioids (tilidin, methadone, EDDP, morphine, codeine, 6-acetylmorphine), benzodiazepines (flunitrazepam, 7-aminoflunitrazepam, diazepam) and THC in oral biofilm has a run time of 15 minutes. The linearity is estimated in the calibration range of 200-1400 pg/mg for amphetamine and morphine (Group 1), 800-2000 pg/mg for THC and 20-800 pg/mg for the other analytes (Group 2). The estimated limits of detection are 5-15 pg/mg (Group 2), 170 pg/mg (Group 1) and under 800 pg/mg for THC. The estimated lower limit of quantification (LOQ) is 15-20 pg/mg (Group 2), 180 pg/mg (Group 1) and 800 pg/mg for THC.

Discussion: Combining this in-vitro model with a highly sensitive LC-MS/MS detection method enables realistic simulation of post-consumption scenarios and serves as the basis for further research of dental biofilm analysis for drugs. The higher number of analytes and low detection limits demonstrate the potential of this approach for future forensic toxicological investigations.

Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

O-059

From Drug Use to Filter: A Mechanistic Framework for Exhaled Breath Aerosol Detection of Drugs of Abuse

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Abstract

Introduction: Exhaled breath aerosols are increasingly used for non-invasive detection of drugs of abuse. We have developed a combined pharmacokinetic and thermodynamic model that traces the pathway from drug use, through plasma, to detection as aerosol in exhaled breath.

Objectives: The objectives of this work were to construct a predictive model that estimates the total aerosol mass collected on a filter while accounting for any mass lost to vapor. The model was designed to be generally useful for understanding aerosol breath collection across a variety of drugs of abuse. Model predictions were compared with published literature data to help explain common observations in exhaled breath aerosol studies.

Methods: The model first determines drug plasma concentration either from direct measurement or dosing information. It then follows the drug of interest from the plasma into the epithelial lining fluid, where aerosol formation occurs. Next, the model accounts for vapor pressure effects to determine the final mass expected on an aerosol filter. All input parameters are taken directly from established primary literature whenever possible.

Results: For drugs like methadone, the model predicts aerosol masses in the range of 25-160 pg depending on dose and volume of breath collected. These values are squarely in the range of experimental observations (11-867 pg) that are reported in Ljungkvist et al. J. Breath Res. 12, 2018, 016011. For others, there are discrepancies where observed filter masses are substantially higher than the model predicts. In some cases, the discrepancies cannot be resolved by reasonable adjustments to core physiological parameters, leaving open the possibility of non-systemic contributions. In total, we provide our initial modelling results for buprenorphine, fentanyl, D9-THC, methadone, tramadol, cocaine, ketamine, heroin, amphetamine, and ethanol.

Discussion: To the best of our knowledge, this is the first mechanistic model that attempts to quantitatively describe the pathway from drug use to collected breath aerosol. The approach offers a novel framework for predicting which drugs of abuse are likely to be readily observed in breath aerosols and which are likely to prove challenging. In addition, because the model predicts only the systemic contributions from epithelial lining fluid to captured aerosol mass, it provides an opportunity to examine the potential impact of non-systemic contributions on observed aerosol masses. Known limitations of the model include uncertainties associated with input parameters, the volume of epithelial fluid collected, and aerosol filter collection efficiencies.

Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

O-060

First Results From Breath Measurements of Acute Cannabis Elimination (BACE)

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Abstract

Introduction: Legal recreational cannabis creates the need for non-invasive chemical determination of recent use. Breath is a promising non-invasive matrix, and peer-reviewed studies have demonstrated that Δ^9-tetrahydrocannabinol (THC) in breath increases immediately after inhalation of cannabis. While industry is developing devices for on-site THC detection in breath, challenges remain to achieving a meaningful measurement that can be deployed in a variety of scenarios (e.g., workplace, roadside). If THC persists in breath after the impairment window, single timepoint measurements are not suitable to determine recent use. Large studies with precisely-timed breath samples are required to investigate whether data analysis methods based on multiple timepoints could be used instead.

Objectives: The first objective of this study is to determine if THC is present in breath samples following self-reported abstinence (≥ 8 h, overnight) from cannabis use. The second objective is to determine the post-use maximum THC concentration and follow the pharmacokinetic decay of THC out to 2 h after cannabis use.

Methods: Forty-five study participants (regular cannabis users) participated in an initial session (clinical setting, no cannabis use) and two experimental sessions (mobile laboratory outside personal residence). Replicate breath samples were collected three times before use, separated by 5 min, and ten non-replicate breath samples after use, separated by 5 or 15 min up to 2 h after use. Breath samples were collected with an impaction filter device with simultaneous spirometry measurements. Liquid chromatography-tandem mass spectrometry was used to positively identify and quantify THC. The accuracy of the analytical method is within ±20% and the lower limit of quantitation (LLOQ) is 10 pg/g; values below the LLOQ are included for data exploration.

Results: 1305 breath samples were analyzed from study participants. More than 80% of participants reported abstinence of 8.5 h to 16 h before the study session; the remaining reported abstinence up to 60 h. Approximately 80% of breath samples collected prior to cannabis use were THC positive despite the reported abstinence, ranging from 0.4 to 200 pg/device (average = 10 pg/device). All breath samples collected after cannabis use were THC positive. Post-use maximums ranged from 20 to 4000 pg/device and post-use minimums ranged from 2 to 200 pg/device.

Discussion: The majority of breath samples had THC that could be positively identified and quantified, including samples collected after at least 8 h of self-reported abstinence. When comparing data between participants, THC concentrations measured before use and after use overlap, demonstrating that a single positive THC measurement does not indicate recent use. Within participant data comparisons are more revealing. By 2 h after use, THC concentrations remain approximately 10 ± 7% of the maximum value of THC seen for each participant. Participants' pre-use THC concentrations were approximately 3 ± 5% of their maximum value.

The study is ongoing and will have a robust statistical analysis to further explore if multiple breath measurements could provide a meaningful indication of recent cannabis use.

Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

O-061

Examining the Placental Transfer of Methamphetamine and Its Influence on the Human Placenta Using ex vivo Human Placenta Perfusion

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Abstract

Introduction: Prescription drugs and drugs of abuse can harm the unborn when used during pregnancy. The only method of investigating the direct transfer of substances through the human placental barrier outside the intrauterine environment is the technique of ex vivo human placenta perfusion. Methamphetamine is a widely abused drug that poses significant risks during pregnancy, including fetal growth restriction, preterm birth, intrauterine fetal death, and placental abruption.

Objectives: While studies in sheep indicate that methamphetamine can cross the placenta, the dynamics of its transfer and its impact on the human placenta are not well understood. This study uses a dual-sided ex vivo placenta perfusion model to investigate the transfer of methamphetamine in human tissue.

Methods: Healthy term placentas from uncomplicated pregnancies were perfused (approved by the ethics committee of the Friedrich-Schiller University 2024-3627-BO-A) for four hours with “maternal” perfusion media containing varying methamphetamine concentrations (50 ng/mL, 200 ng/mL, and 800 ng/mL), along with control perfusions without methamphetamine (each group n=3). Perfusion media samples were collected from both “maternal” and “fetal” sides at regular intervals. Throughout the experiment the following parameters were monitored: transfer control (antipyrine and creatinine) concentrations as well as glucose and lactate concentrations on both sides, pressure on the fetal side, and maternal ß-hCG concentration. Methamphetamine, its metabolite amphetamine, and the transfer controls were simultaneously analyzed using a validated liquid chromatography (LC) - high resolution mass spectrometry (HRMS) method to minimize sample volume, time and cost. For this approach, 50 µL sample was precipitated with 250 µL acetonitril:methanol (4:1) containing internal standard and diluted with mobile phase. LC separation was performed using gradient elution on a Nucleoshell RP 18 plus column. Analytes were detected using HRMS after electrospray ionization in positive mode. A seven-point calibration model (1 to 1000 ng/mL for amphetamine and methamphetamine in both fetal and maternal perfusion media) and quality control samples (20 ng/mL and 800 ng/mL in fetal and maternal media) were used.

Results: The data showed that methamphetamine crosses the placenta within 30 minutes, reaching a dose-dependent equilibrium, while no metabolism to amphetamine occured. Mean equilibrium concentrations were 15.1 ng/mL (fetal) and 15.8 ng/mL (maternal) after 4 hours (50 ng/mL), 76.9 ng/mL (fetal) and 80.2 ng/mL (maternal) after 2 hours (200 ng/mL). In the 800 ng/mL perfusion experiment, equilibrium had not been reached after 4 hours with mean concentrations of 287 ng/mL (fetal) and 322 ng/mL (maternal) being observed. Although the metabolic function of the placenta does not appear to have been impaired by the administration of a single dose of methamphetamine, there is a tendency towards reduced ß-hCG production in its presence.

Discussion: This is the first time, where the ex vivo study demonstrates that methamphetamine rapidly and, in a dose-dependent manner crosses from the maternal to the fetal circulation. While placental metabolism does not appear to be significantly impacted by a single exposure, reduced production of ß-hCG may be associated with the known risks of methamphetamine use during pregnancy. These findings highlight the urgent need to raise awareness of the risks of methamphetamine use during pregnancy.

Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

O-062

Quantification of GLP-1 Receptor Agonists Semaglutide and Tirzepatide as Well as Insulin Variants Degludec and Icodec in Postmortem Vitreous Humor Using a Combined LC-MS/MS Method

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Abstract

Introduction: The postmortem analysis of proteins remains challenging due to instability in hemolyzed blood and low concentrations in alternative matrices. Since medication errors as well as suicides and homicides involving insulins can result in life-threatening hypoglycemia, reliable MS-based methods for insulin variants are required in forensic toxicology. Meanwhile, glucagon-like peptide-1 receptor agonists (GLP-1 RA) represent a rapidly expanding class of therapeutic peptides, with emerging reports of adverse effects and counterfeit products, some containing insulins instead of GLP-1 RA. Despite this, postmortem MS data remain scarce for insulins and GLP-1 RA.

Objectives: This study aimed to extend a previously developed SPE-LC-MS/MS method for insulin variants to include GLP-1 RA and to evaluate the detectability of therapeutic GLP-1 RA concentrations in postmortem matrices. The method was applied to vitreous humor (VH), cerebrospinal fluid (CSF) and femoral blood from two cases. Case 1 involved therapeutic semaglutide use discontinued two weeks prior to death. The 52-year-old diabetic was also treated with insulin lispro and degludec and had been hospitalized for hyperglycemia one day prior to death. In case 2, a 47-year-old diabetic allegedly injected 7.5 mg of tirzepatide before developing anxiety and nausea and dying the next morning. In both cases, the cause of death remained unclear after autopsy and routine toxicological screening.

Methods: The method covers C-peptide, 11 insulins (human, lispro, aspart, glulisine, glargine, glargine metabolite M1, degludec, detemir, icodec, porcine and bovine (internal standard)) and 8 GLP-1-related analytes (GLP-1(72-108), GLP-1(72-107 amide), exenatide, lixisenatide, liraglutide, semaglutide, tirzepatide and retatrutide). Sample preparation included protein precipitation followed by a mixed-mode anion exchange micro-elution SPE (conditioning with ACN, equilibration with diluted NH_4OH, loading, washing with diluted NH_4OH and ACN/H2O/NH_4OH and elution with ACN/H2O/AcOH). Chromatographic separation was achieved using a YMC Accura Triart Bio C18 column (1.9 µm, 2.1×150 mm, 300 Å). LC-MS/MS analysis was performed using an Agilent 1290 Infinity II LC and an Agilent 6495 triple quadrupole MS. Data were processed using MassHunter Quantitative Analysis.

Results: In case 1, semaglutide and insulin degludec were detected in VH at 0.45 ng/mL and approximately 0.14 ng/mL. In case 2, tirzepatide was quantified at 1.7 ng/mL in VH, alongside approximately 15 ng/mL of insulin icodec. In CSF, concentrations were 6.4 ng/mL (tirzepatide) and 2.1 ng/mL (icodec). Quantification was based on calibration in pooled negative postmortem VH, CSF or plasma over a range of 0.25 - 10 ng/mL.

Discussion: Published data on semaglutide, tirzepatide and icodec in postmortem specimens are lacking and insulin degludec has only been described in postmortem tissues at comparatively high detection limits. The absence of semaglutide, degludec and icodec in the blood samples

supports limited stability in hemolyzed samples, consistent with previous observations for insulins. Quantification of semaglutide in VH despite a prolonged post-administration interval demonstrates both method sensitivity and analyte persistence in this matrix. These findings highlight the importance of alternative matrices for the detection of therapeutic proteins. Due to the lack of postmortem reference data, interpretation of the measured concentrations remains limited, however, the findings in case 2 could support insulin icodec overdose as a possible cause of death.

Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

O-063

Postmortem Skeletal Toxicology: A Fresh Approach to a Persistent Problem

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Abstract

Introduction: Tissues routinely used for postmortem toxicology offer only a snapshot of exposure(s) in the days, hours, and minutes leading to death. Furthermore, many substances are not routinely tested for. Postmortem toxicology using bone tissue has the potential to enhance the epidemiology of substance misuse.

Objectives: The objectives of this presentation are to: 1) Describe a method for detecting drugs/drug metabolites from human rib bone, 2) Demonstrate substances that are detectable from rib bone of a decedent compared to those from blood sampled at autopsy, and 3) Discuss the usefulness of bone as a matrix for postmortem toxicology.

Methods: This study is based on n=119 rib bone and blood samples collected from decedents receiving a postmortem exam at the Western Michigan University Homer Stryker MD School of Medicine, Office of the Medical Examiner from 2021 to 2023. Both sample types were analyzed via Liquid Chromatography Tandem Mass Spectrometry (LC-MS/MS). To ensure that soft tissues did not confound the analysis, ribs were manually cleaned of adhering soft tissue, marrow was rinsed out of the trabecular bone, and the samples were cut into small pieces no larger than 1.2-x-2.0-cm. Samples weighing 2.0 g (+/- 0.1 g) were washed in a 50:50 methanol:saline solution, vortexed and centrifuged. The supernate was drawn off, and the procedure repeated a second time. Samples were then subjected to a third wash in sodium phosphate buffer solution, vortexed, and centrifuged. The supernate was drawn off, and the samples were then vortexed in acetone. The bone samples were then dried under a fume hood. Once dry, a target of 1.0 g (+/- 0.1 g) of bone was placed into 7.0 mL lysing tubes with 9.0 g (+/- 0.2 g) of steel beads (Bertin Instruments, Montigny-le-Bretonneux, France). The lysing tubes were loaded into a Precellys Evolution Homogenizer programmed to perform six 20-second cycles at 6500 rpm with a 30-second pause between cycles. Solid-phase extraction for analysis by LC-MS/MS was performed after the wash protocol. The LC-MS/MS analysis targeted 49 drugs of abuse and metabolites.

Results: A total of n=25 (51.0%) substances were detected in rib bone. Interestingly, n=9 (18.4%) of the substances, including opioids, amphetamine, benzoylecgonine, mitragynine, and xylazine, were detected in the rib bone but not in autopsy blood from the same decedent.

Discussion: Drugs of abuse that were detected in bone tissue, but not in autopsy blood, were generally consistent with the reported drug-use behavior(s) of the decedent and suggest that bone provides a longer record of substance exposures. The majority of epidemiological studies characterizing drug-related mortality are based on acute exposure(s) and are likely missing important data. While postmortem skeletal toxicology research is in its infancy, it deserves serious attention and has the potential to make significant contributions to the study of drug-related morbidity and mortality. Postmortem skeletal toxicology provides information that is interpretable and useful for understanding cause of death for the individual, as well as epidemiological trends in regional drug-related morbidity and mortality.

Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

O-064

Broad-Scope Alternative Matrix Method: Development and Validation of LC–QTOF-MS Method for Detection of 946 Drugs and Metabolites in Postmortem Tissue

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Abstract

Introduction: In postmortem toxicology and fatal driving under the influence of drugs (DUID) cases where blood or urine are unavailable, alternative biological matrices are required for analysis. A tissue-based method was developed to enable in-house forensic toxicology testing and expand the scope of the San Francisco Office of the Chief Medical Examiner's existing liquid chromatography–quadrupole time-of-flight mass spectrometry (LC-QTOF-MS) workflow.

Objectives: This study describes the development and validation of a qualitative LC-QTOF-MS method using Sequential Window Acquisition of All Theoretical Fragment-Ion Spectra (SWATH) for the detection and confirmation of 946 drugs and metabolites in postmortem liver, spleen, psoas muscle, gastric contents, and brain.

Methods: For each matrix, 1 g of specimen was diluted with 4 mL of Millipore water and two stainless steel grinding balls in a screw-top plastic jar. The tissue was then homogenized using a Spex SamplePrep Geno/Grinder 2010. The homogenate was subjected to protein precipitation followed by size-exclusion filtration, consistent with existing developed, validated, and routinely implemented methodologies. Method validation was performed in accordance with ANSI/ASB Standard 036.

Results: The method reliably detected 946 analytes across 35 drug classes in all five matrices and demonstrated robust performance in accordance with ANSI/ASB Standard 036.

No significant carryover was observed for 98% of analytes across all matrix types. Median matrix effects ranged from 0–20% across matrices; however, 74%-83% of analytes exceeded the ±25% threshold at either low or high concentrations, reflecting the inherent complexity of tissue homogenates. Matrix interferences were observed in approximately 6–10% of analytes, with the highest frequency in liver and muscle and the lowest in brain. Reliable identification was maintained for 942, as interfering peaks did not satisfy established identification criteria. Analyte identification was based on published identification criteria utilizing a combined weighted score incorporating mass error, isotope ratio, library match, and relative retention time.

Processed sample stability was maintained for over 18 hours, corresponding to a typical analytical run, with consistent identification. The median limit of detection was 2.25 ng/mL; 80% of analytes were ≤ 10 ng/mL and 90% were ≤ 30 ng/mL, demonstrating high sensitivity and reliable qualitative identification. Extraction using protein precipitation with size-exclusion filtration produced the cleanest extracts in brain tissue, followed by spleen, liver, muscle, and gastric contents. Sensitivity trends aligned with extract cleanliness, with the highest responses observed in brain and spleen.

Drug identifications from 16 authentic case samples in blood and urine were compared to corresponding tissue samples, demonstrating a high degree of detection agreement between matrices. These findings support the use of tissue analysis both as a complementary tool alongside traditional blood and urine testing and as a suitable alternative when conventional matrices are unavailable.

Discussion: These findings demonstrate the feasibility of a large-scope LC-QTOF-MS method in tissue matrices, addressing a critical gap in toxicological analysis. The expanded analyte panel across five matrix types enables comprehensive drug detection in the absence of traditional specimens. Implementation of this method will reduce turnaround time in cases requiring tissue analysis by enabling in-house testing and eliminating delays associated with outsourced analysis, while also lowering costs and enhancing analytical coverage in routine forensic casework.

Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

O-065

Detecting Prenatal Alcohol Exposure Using PEth in Dried Blood Spots and LC-MSMS

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Abstract

Introduction: Prenatal alcohol exposure (PAE) can negatively affect fetal development. Accurate assessment of alcohol consumption is essential for monitoring exposure and evaluating interventions during pregnancy. Phosphatidylethanol 16:0/18:1 (POPEth) and 16:0/18:2 (PLPEth) are commonly used to assess drinking history. Evidence suggests that PLPEth forms and is eliminated more rapidly than POPEth; therefore, the POPEth/PLPEth ratio may provide additional insight into the timing and pattern of alcohol consumption.

Objectives: To monitor prenatal alcohol exposure in high-risk pregnant women by quantifying POPEth and PLPEth concentrations and evaluating their ratio using liquid chromatography-mass spectrometry (LC-MS/MS) in dried blood spots (DBS).

Methods: DBS samples were collected from high-risk pregnant women (n=596) across three clinical visits during pregnancy at UTHealth in Houston, TX. Samples were analyzed using a validated LC-MS/MS method (linearity range of 5–800 ng/mL) developed in accordance with ANSI/ASB 036 and DBS-specific recommendations from the official International Association for Therapeutic Drug Monitoring and Toxicology guidelines. A single 10 mm punch was extracted with methanol containing internal standards, followed by centrifugation, evaporation, and reconstitution. Separation was performed using a C18 column with gradient elution. Analysis was performed via LC-MS/MS in negative ion mode with two MRM transitions per analyte.

Results: Of 596 DBS samples from high-risk pregnant women, 438 were positive for POPEth and 477 for PLPEth. Mean ± SD concentrations were 54.1±112 ng/mL for POPEth, 57.3±110 ng/mL for PLPEth. Based on the current consensus on threshold and decision limits for POPEth (Luginbuhl et al., 2021), 11% of the samples were consistent with heavy alcohol consumption (POPEth ≥200 ng/mL), 27% with significant alcohol consumption (POPEth ≥20 but <200 ng/mL), and 62% with light or no alcohol consumption (POPEth <20 ng/mL).

Among the 596 analyzed samples, 149 participants completed all three visits (n=447 DBS samples) and were grouped by visit for analysis. Across the visits, most participants exhibited non-monotonic POPEth trajectories, indicating variability in alcohol consumption. Only 19% of participants had a consistent decrease in PEth concentration across all visits, but overall, 56-57% showed a decrease by the third visit.

The POPEth/PLPEth ratio averaged 0.8±0.5 (range between 0.2 and 3.6), with most values clustered between 0.6 and 1.1. Approximately 12% of the samples had a ratio >1.2, 38% had a ratio between 0.8 and 1.2, and 50% had a ratio <0.8.

Discussion: Application of a DBS- LC-MS/MS method to authentic samples revealed a dynamic pattern of alcohol consumption over time. Notably, 38% of the samples were consistent with significant or heavy alcohol consumption. POPEth/PLPEth ratios centered around 0.7 and 0.8, indicating a relatively consistent relationship between the two analytes across the samples;

however, higher variability was observed at low POPEth concentrations. 50% of samples showed a ratio <0.8, indicating higher PLPEth values and therefore suggesting a more recent exposure. Assessments of POPEth concentration, in conjunction with POPEth/PLPEth, may provide additional insight into drinking behavior patterns and help evaluate the effectiveness of interventions during pregnancy.

Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

O-066

Comparison of POPEth, PLPEth, Ethyl Glucuronide, and Ethyl Sulfate Detection Across Biological Matrices

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Abstract

Introduction: Ethanol, a commonly encountered drug for forensic toxicologists, is rapidly eliminated from the bloodstream in under 12 hours. Direct biomarkers, ethyl glucuronide (EtG), ethyl sulfate (EtS), and phosphatidylethanol (PEth), can extend the detection window of alcohol consumption, with PEth providing the longest window (2-4 weeks). PEth can be measured in whole blood (WB) or dried blood spots (DBS), with DBS offering advantages in sample collection, storage, and stability.

Objectives: Compare PEth 16:0/18:1 (POPEth) and PEth 16:0/18:2 (PLPEth) in WB and DBS, and EtG/EtS in oral fluid and urine, for detection of ethanol exposure.

Methods: Participants enrolled in an IRB-approved study investigating alcohol biomarkers (n=22) donated WB, DBS, oral fluid, and urine samples at 16 collection time points over approximately 2 months. DBS samples were collected using HemaXis 10-µL volumetric DBS cards, WB in 6-mL lavender-top tubes, oral fluid using Quantisal® collection devices, and urine in specimen cups without preservative. DBS samples were stored at room temperature, while WB, oral fluid, and urine were refrigerated until analysis. One 10-mm hole punch from DBS HemaXis cards (n=246) was extracted in one mL of methanol for 90 minutes in the presence of the internal standards (POEth-d_5 and PLEth-d_5, 20µL at 0.1µg/mL), followed by centrifugation, evaporation, and reconstitution with 200µL acetonitrile:methanol (90:10, v/v). The analysis was achieved by LC-MS/MS in negative ESI mode with an analytical range of 5-800ng/mL. WB specimens (75µL) were diluted with 500µL of PEth isotopically labeled internal standards in acetonitrile:isopropanol (50:50, v/v) to precipitate proteins. Following centrifugation, the supernatant was filtered and diluted for injection on a validated LC-MS/MS method with an analytical range of 10-400ng/mL. Oral fluid samples were prepared with solid phase extraction and quantified by a validated LC-MS/MS method, with EtS linear range of 12.5-2500ng/mL in neat oral fluid. Urine samples were screened by EtG immunoassay (500ng/mL) and confirmed with a validated LC-MS/MS method reporting EtG 500-10,000ng/mL and EtS 100-10,000ng/mL. All validations followed the ANSI/ASB 036 Standards.

Results: POPEth was detected in 209/267 DBS samples (78.3%), with a mean concentration of 42.7ng/mL (5.2-223.5ng/mL), while PLPEth was detected in 214/267 samples (80.1%), with a mean concentration of 40.7ng/mL (4.4-209.1ng/mL). In WB, POPEth was detected in 62/303 samples (20.5%), with a mean concentration of 38.9 ng/mL (10.6-198ng/mL), and PLPEth in 64/303 samples (21.1%), with a mean of 37.8 ng/mL (10.5-259.4ng/mL). Among evaluated oral fluid samples (n=308), EtS was detected in only one sample (14.5ng/mL). In urine, EtG and EtS were detected in 10 of 305 samples.

Discussion: Among the four matrices evaluated, DBS showed the highest detection frequency. Among paired samples (n=261), DBS and WB agreed in 106 samples (51 detected in both and 55 not detected in either), while 155 cases were detected in DBS (with concentrations of 5-125ng/mL for both PEths) but not in WB. PEth measured in DBS and WB demonstrated substantially higher frequencies than EtS in oral fluid and EtG/EtS in urine. PEth measured in DBS demonstrated the highest detection frequency among the matrices evaluated.

Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

O-067

Pediatric Intoxication to the Synthetic Cannabinoid 5F-ADB via Vaping Devices: An Alarming Case Series

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Abstract

Introduction: The use of vaping devices among teenagers increasingly involves cannabinoids known as 'Buddha Blue,' 'Spleen,' or 'PTC.' The synthetic cannabinoid 5F-ADB (5F-MDMB-PINACA) is frequently identified in these devices and has been linked to serious complications, including fatalities. Although 5F-ADB exhibits a toxicological profile similar to that of cannabis, its potency is 290 times greater than that of delta-9-tetrahydrocannabinol. Furthermore, spiking vaping liquids with unknown doses of this compound significantly increases the risk of acute toxicity. Notably, exposure may also occur without the user's knowledge.

Objectives: This study describes a cluster of pediatric poisoning cases associated with the use of 5F-ADB via vaping devices, characterizing the primary clinical manifestations and their corresponding quantified biological concentrations.

Results: Between April 2025 and mid-February 2026, 39 poisonings, including three recurrences, involving 5F-ADB consumption were documented via toxicological analyses at Grenoble Alpes University Hospital (France). 5F-ADB or one of its metabolites were first identified in plasma and/or urine following targeted screening for Novel Psychoactive Substances, performed using liquid chromatography coupled with high-resolution mass spectrometry (LC-HRMS). Separation was achieved on an Accucore Phenyl-Hexyl column (2.1 mm x 100 mm, 2.6 µm) using an Orbitrap Exploris 120 mass spectrometer and an inclusion list from the HighResNPS database. Subsequently, a quantification method using liquid chromatography-tandem mass spectrometry (LC-MS/MS) was developed for 5F-ADB (m/z 378>233 and 378>318) and two of its metabolites, the hydroxy-pentyl C20H29N3O4 designated as « M2 » (m/z 376>213 and 376>145) and the carboxylic acid C19H26FN3O3 designated as « M7 » (m/z 364>233 and 364>145). Separation was achieved on an Acquity HSS C18 column (2.1 mm x 150 mm, 1.8 µm) using an Xevo TQ-XS mass spectrometer. The limit of quantification (LOQ) was determined at 0.10 ng/mL, linearity, carry-over, intra- and inter-day precision and accuracy were verified.

The median age of the patients was 15 years (range: 12-17 years), with a male-to-female ratio of 3:1. In 53% of cases, 5F-ADB was detected at trace levels in plasma (< LOQ). However, the M7 metabolite exhibited higher concentrations in both plasma (0.2–254 ng/mL) and urine (0.3–730 ng/mL) compared to the M2 metabolite (plasma: 0.1–1.2 ng/mL; urine: 0.2–26 ng/mL), making M7 a biomarker of interest for identifying 5F-ADB use. The predominant clinical presentations observed were neurological (n=24) and cardiovascular (n=10), characterized primarily by impaired consciousness, chest pain, and tachycardia; however, no significant correlation with biological concentrations was found. All patients were admitted to the

emergency department and 12 were hospitalised for at least one day, including one in intensive care. According to patient reports, 50% were chronic users of 5F-ADB.

Discussion: An increase in 5F-ADB cases has been observed in the French Alps, highlighting the critical need for accurate identification to optimize both clinical management and addiction care. The M7 metabolite has been established as a primary biomarker for detecting exposure to this specific cannabinoid. Given its high prevalence, it is essential to update the databases used in toxicological screening. According to recent data from the Center for Forensic Science Research & Education, 5F-ADB is also one of the most frequently detected synthetic cannabinoids in the United States in 2026.

Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

O-068

Toxicokinetic Profiling and Metabolic Characterization of MDMB-4en-PINACA in Zebrafish (*Danio Rerio*) Using LC-MS/MS and LC-HRMS

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Abstract

Introduction: The rapid emergence of new psychoactive substances (NPS), particularly synthetic cannabinoid receptor agonists (SCRAs), poses significant challenges for clinical and forensic toxicology. Despite their widespread use, data on toxicokinetics and metabolism remain limited. The zebrafish (*Danio rerio*) model has emerged as a promising *in vivo* system for integrated toxicological assessment, enabling a preliminary approach for assessing internal exposure, key biotransformation, and time-dependent effects.

Objectives: This study aimed to characterize the toxicokinetic profile and metabolic pathways of the SCRA MDMB-4en-PINACA in zebrafish, integrating internal concentration measurements and metabolite identification.

Methods: Zebrafish larvae (5 dpf) were exposed to MDMB-4en-PINACA at 10 µM. Uptake was evaluated at 0.25, 0.5, 1, 2, 4, and 8 h of exposure, whereas elimination was assessed at 0.25, 0.5, 1, 2, 4, 6, 24, and 48 h after transfer to clean medium. Tissue distribution was evaluated in triplicate using pools of 16 larvae per time point and assessed by quantifying MDMB-4en-PINACA in head, trunk, and tail sections collected after 8 h of exposure and after 48 h of depuration in clean medium. Internal concentrations were determined using a previously developed LC-MS/MS method. Toxicokinetic parameters were estimated using a two-step approach, with terminal elimination constant obtained from the log-linear phase and subsequent fitting of the uptake phase with fixed $k_{\_out}$. One- and two-compartment toxicokinetic models were compared using the corrected Akaike Information Criterion (AICc) to evaluate model fit and complexity. For metabolic characterization, sample extracts were analyzed by LC-HRMS before and after enzymatic hydrolysis using B-One® β-glucuronidase (Kura Biotech), allowing the assessment of glucuronide metabolites in addition to non-conjugated phase I metabolites. Metabolite identification was based on exact mass, retention time, and MS/MS fragmentation patterns.

Results: MDMB-4en-PINACA showed rapid uptake and measurable persistence in zebrafish larvae. The terminal elimination phase (6–48 h) was approximately monoexponential, with $k_{\_out}$ = 0.0394 h^{-1}, $t_{1/2}$ = 17.6 h, and R^2 = 0.887. Uptake fitting yielded $k_{\_in}$ = 25.44 $L{\cdot}kg^{-1}{\cdot}h^{-1}$ and BCF ≈ 646 $L{\cdot}kg^{-1}$, indicating efficient absorption and significant bioconcentration. Although both one- and two-compartment models adequately described the uptake phase, the two-compartment model better captured the early elimination profile, supporting an initial redistribution phase after transfer to clean medium. Metabolic analysis revealed extensive biotransformation, with 18 metabolites identified. Major phase I pathways included ester hydrolysis, N-dealkylation, hydroxylation, and dihydrodiol formation, and sequential oxidation of the 4-enyl side chain, followed by phase II glucuronidation.

Discussion: Zebrafish larvae provided an effective *in vivo* platform for the integrated characterization of MDMB-4en-PINACA toxicokinetics and metabolism. The compound showed rapid uptake, relevant bioconcentration, prolonged persistence, and extensive phase I/II biotransformation. These findings reinforce the applicability of zebrafish for mechanistic and translational studies of high-potency SCRAs.

Acknowledgments

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Disclosure

Yes, I, or a member of my immediate family, has a financial interest to disclose.

Conflict of Interest

Grant/Research Support: the authors declare no competing financial interests related to this study. Travel support was provided through a grant from Kura Biotech.

O-069

Pharmacological Characterization of Hexahydrocannabinol-Derived Semi-Synthetic Cannabinoids at the Cannabinoid Type 1 Receptor Using a β-Arrestin2 Recruitment Assay

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Abstract

Introduction: Semi-synthetic cannabinoids (SSCs) represent a rapidly expanding subclass of cannabinoids on the recreational drug market, driven by the demand for 'legal' Δ^9-tetrahydrocannabinol (Δ^9-THC) alternatives. These compounds arise from structural diversification of either the Δ^9-THC or cannabidiol (CBD) scaffold. Hexahydrocannabinol (HHC), among the first SSCs to gain prominence, is obtained via hydrogenation of the C_9–C_{10} double bond of Δ^9-THC, yielding 9(R)- and 9(S)-diastereomers. Following regulatory control, structurally modified HHC derivatives have emerged, including compounds with a modified C_3 side chain or substitution of the C_1 hydroxyl group with an ester. Despite their increasing prevalence, their pharmacological properties remain poorly characterized.

Objectives: Determination of the intrinsic cannabinoid type 1 receptor (CB1) activation potential of fourteen emerging HHC-derived SSCs.

Methods: CB_1 activation was assessed with a CB_1/β-arrestin2 recruitment assay using split nanoluciferase complementation as a read-out. Δ^9-THC and the synthetic cannabinoid CP55,940 were included as reference compounds. Pharmacological characterization enabled determination of potency (EC_{50}; concentration producing the half-maximal effect), and efficacy (E_{max}; maximal achievable effect). The panel comprised 9(R)-and (9)-HHC derivatives, including C_1 ester derivatives (9(R)- and 9(S)-HHC-O-acetate and 9(R)- and 9(S)-HHCP-O-acetate), and C_3 side chain variants with butyl up to nonyl tails: HHCB (C4), HHCH (C6), HHCP (C7), HHC-C8 (C8), and HHC-C9 (C9). All compounds were evaluated as individual epimers, with HHC (C5) included for structure–activity relationship (SAR) assessment. To minimize compound adsorption due to high lipophilicity, an optimized protocol was followed. Stability of the C_1-esters was assessed using LC-HRMS/MS.

Results: Compounds were classified as exhibiting: (i) no or lower, (ii) comparable, or (iii) higher CB_1 activation potential than Δ^9-THC. The first category included 9(S)-HHC and several derivatives; 9(S)-HHC-O-acetate and 9(S)-HHCB showed no activity at CB_1, while 9(S)-HHCP-O-acetate displayed strongly reduced potency. 9(R)-HHCB was the only compound in the second category, showing activity comparable to Δ^9-THC. All remaining compounds fell into the third category, exhibiting increased efficacy but reduced potency relative to Δ^9-THC. 9(R)-HHC and its ester derivatives displayed a 2–3-fold increase in efficacy relative to Δ^9-THC, although esterification reduced potency. LC–HRMS/MS confirmed absence of hydrolysis under assay conditions. Also C_3 chain-elongated derivatives displayed increased efficacies, most notably for the C8 and C9 derivatives, which displayed efficacies up to 6–20-fold higher than Δ^9-THC, and approached or even surpassed that of CP55,940.

Discussion: Elongation of the C_3 side chain resulted in increased efficacy (C5 < C6 < C8 < C9), with reduced potency (C5 > C6 > C8 > C9), except for the C7 derivatives, which showed higher potency but lower efficacy than the corresponding C6 derivatives. The high efficacy of the C8–C9 derivatives may raise toxicological concern; however, their reduced potency suggests such effects may require concentrations unlikely to be attained in humans. C_1-esterification either abolished CB_1 activation potential or markedly reduced potency; hydrolysis was excluded *in vitro*, although *in vivo* hydrolysis remains likely, releasing the more bioactive C_1-OH counterparts. Collectively, these findings demonstrate that minor structural modifications can markedly alter CB_1 activation potential, supporting improved risk assessment and monitoring of SSCs.

Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

O-070

Structure-Activity Relationships of ADB-HEXINACA Analogues and Their Metabolites at the CB1 Receptor

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Abstract

Introduction: Synthetic cannabinoid receptor agonists (SCRAs) are potent and efficacious agonists of cannabinoid receptor type 1 (CB_1), designed to mimic Δ^9-tetrahydrocannabinol (Δ^9-THC). However, unlike Δ^9-THC, a partial CB_1 agonist, SCRAs are full agonists capable of causing more serious toxic effects. There is extensive data on CB_1 activity for many parent compounds, but only a limited number of studies have assessed the CB_1 activity of SCRA metabolites. ADB-HEXINACA was the first SCRA incorporating a hexyl tail moiety to be detected on the illicit drug market in 2021, and the continual structural evolution of SCRAs means that additional hexyl tail analogues may emerge in future. *In vitro* and *in vivo* metabolite identification studies have identified a unique ketone metabolite, a 5-oxo-hexyl product, as a major metabolite of ADB-HEXINACA, along with a 5-hydroxy-hexyl metabolite and their corresponding terminal amide hydrolysis products. However, no CB_1 activity data for these metabolites – or indeed for any SCRA ketone metabolite – have been reported to date.

Objectives: The aim of this study was to characterise the CB_1 activity of ADB-HEXINACA and its structural analogues (MDMB-HEXINACA, AB-HEXINACA and ADB-HEXICA). The 5-oxo-hexyl, 5-hydroxy-hexyl and their corresponding terminal amide/ester hydrolysis biotransformation products were also assessed to determine how metabolism of hexyl tail SCRAs affects CB_1 activity.

Methods: *In vitro* CB_1 activity was assessed using the AequoScreen® assay, which measures Ca^{2+} flux following receptor activation. Concentration-response curves (8 points, 29 pM to 60 µM) were generated in triplicate, from which potency (EC_{50}) and efficacy (E_{max}) were calculated. Responses were normalised to the reference compound JWH-018, defined as having 100% activity. A minimum of three independent experiments were conducted for each compound.

Results: Parent SCRAs were high-potency agonists, with EC_{50} values ranging from 41.5 nM for ADB-HEXINACA to 139 nM for AB-HEXINACA, compared with 111 nM for the reference compound JWH-018. Efficacies spanned 133% for ADB-HEXICA to 147% for MDMB-HEXINACA. Oxidation of parent compounds to 5-oxo-hexyl metabolites resulted in products that retained substantial CB_1 potency and efficacy, while the 5-hydroxy-hexyl metabolites showed similar efficacy but lower potency. The corresponding terminal amide/ester hydrolysis products displayed low or undetectable CB_1 activity.

Discussion: These *in vitro* data are the first to characterise the CB_1 activity of SCRA ketone metabolites and indicate that these biotransformation products are toxicologically relevant, likely contributing to the pharmacological effects and risk profiles of their parent SCRAs *in vivo*. 5-Hydroxy-hexyl metabolites, though lower potency, may also contribute, while amide/ester hydrolysis of both the ketone and hydroxy metabolites inactivates them. These findings highlight that specific metabolic pathways influence CB_1 activity and, therefore, SCRA toxicity.

Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

O-071

Analysis of Phytocannabinoids and Semisynthetic Cannabinoids From Postmortem Blood Samples Using Gas Chromatography-Tandem Mass Spectrometry (GC-MS/MS)

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Abstract

Introduction: A large variety of new semisynthetic cannabinoids (SSC) has emerged into markets in recent years. EUDA (European Union Drug Agency) is currently monitoring 37 different SSCs, of which some has been classified as controlled substances in many countries. SSCs form an analytical challenge as they are structurally related to each and the total number of SSCs available as reference material is already over 170. Most of them possess also stereoisomers. The concentrations of SSCs can be rather low in biological samples, thus high specificity and sensitivity are required.

Objectives: The objective of the study was to develop and validate a comprehensive method for the analysis of the common phytocannabinoids and semisynthetic cannabinoids from postmortem blood samples using GC-MS/MS.

Methods: The method utilizes liquid-liquid extraction (LLE) with hexane:ethylacetate (5:1), derivatization with MSTFA, and analysis with GC-MS/MS (Agilent 8890/7010D using an HP-5Q ultra-low-bleed column). 46 cannabinoids are analyzed using six isotopically labelled cannabinoids as internal standards. The calibration is based on matrix-matched calibrators using bovine blood. The method was validated using a postmortem blood as the test matrix.

Results: A total of 46 phytocannabinoids and semisynthetic cannabinoids is analyzed from postmortem blood samples. The method was fully validated (specificity, selectivity, calibration model, lower and upper limit of quantitation, repeatability, reproducibility, bias, matrix effects, carry-over, and measurement uncertainty). The lower limit of quantitation varies between 1 and 3 µg/L and the upper limit of quantitation is 150 µg/L. The calibration is based on a quadratic regression model with $1/x^2$ weighting. The measurement uncertainty varies between 10 and 45%, the average being 22%.

Discussion: A novel analytical method was developed for phytocannabinoids and semisynthetic cannabinoids from postmortem blood samples using GC-MS/MS. The analytical run lasts for 10 minutes and all analytes could be separated from each other by retention time and/or specific ions. A slower temperature program (run time 20 min) enables the separation of stereoisomers, such as 9(S)-hexahydrocannabiphorol acetate and 9(R)-hexahydrocannabiphorol acetate, if needed. The hydrolysis with β-glucuronidase was also tested for blood samples, as some of the compounds, such as trans-11-nor-9-carboxy-delta-9-THC, can exist in the blood also as glucuronide conjugates. However, it was noticed that cannabinoid esters, such as acetates and butanoates, degraded partially to their native compounds. Thus, hydrolysis is not suitable for blood samples. A slight matrix effect was detected for some compounds, but this effect was compensated using bovine blood for the calibration. In real samples nine different cannabinoids have been detected so far, including e.g. hexahydrocannabinol (HHC), 11-nor-9(R)-carboxy-HHC, delta-9- tetrahydrocannabivarinic

acid, and trans-11-nor-9-carboxy-delta-8-THC. The method can easily be expanded for urine samples as well for clinical samples. Furthermore, new analytes can be added to the method whenever needed.

Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

O-072

Bioisosteres - In Vitro Hepatic Metabolism of Six Cathinone Related Methylthiophene Stimulants

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Abstract

Introduction: Cathinones are potent stimulants, structurally related to amphetamines, and often abused. As indirect sympathomimetics, they increase dopamine and noradrenaline levels. Therapeutic derivatives of natural cathinone such as bupropion (antidepressant) or amfepramone (appetite suppressant) are known. They were partially removed from the market due to severe side effects. To prevent such side effects while maintaining the therapeutic profile, bioisosteric replacement of functional groups is common. The methylthiophenes represents such a group of bioisosteres, in which the phenyl group was replaced by a methylthiophene moiety.

Objectives: This study investigated the in vitro hepatic metabolism of six novel methylthiophene analogues, 5-methyl-*N*-methyl-butanothiophenone (5Me-NM-BT), 5-methyl-*N*-methyl-valerothiophenone (5Me-NM-VT), 5-methyl-*N*-ethyl-butanothiophenone (5Me-NE-BT), 5-methyl-*N*-ethyl-valerothiophenone (5Me-NE-VT), 5-methyl-α-pyrrolidino-butanothiophenone (5Me-αP-BT), and 5-methyl-α-pyrrolidino-valerothiophenone (5Me-αP-VT). Although no reports of human consumption currently exist, likely due to a lack of analytical reference data, this study aimed to identify key metabolic biomarkers. These findings will help in establishing reliable targets for toxicological urine screening.

Methods: For qualitative investigation of hepatic metabolism, pooled human liver S9 fraction (pHLS9) containing cofactors for most phase I and II metabolic reactions was used with an incubation period of one and six hours. Monooxygenases activity screening was performed using eleven recombinant human isoenzymes for 30 minutes. Samples were analyzed by liquid chromatography coupled to high-resolution tandem mass spectrometry.

Results: Across all six methylthiophene analogues, hydroxylation was identified as most frequent phase I reaction. In addition, for the four aliphatic analogues 5Me-NM-BT, 5Me-NM-VT, 5Me-NE-BT, and 5Me-NE-VT, in vitro phase I metabolism was characterized by carboxylation, N-dealkylation, as well as combinations of the aforementioned. In the case of the two α-pyrrolidine analogues, 5Me-αP-BT and 5Me-αP-VT, dihydroxylation, and hydroxylation followed by ring opening of the pyrrolidine ring was observed. The isozyme mapping revealed the contribution of various isozymes including CYP1A2, CYP3A4, CYP2D6, CYP2C19, and CYP2B6.

Discussion: Overall, the investigated compounds exhibited highly similar metabolic pathways, although structural variations resulted in minor differences in isoenzyme preferences. The contribution of several isozymes to their metabolism minimizes the risk of drug-drug-interactions. Hydroxylation was consistently identified as a major metabolic route across all compounds. Consequently, hydroxylated metabolites may represent suitable analytical targets for human urine screening, in addition to the corresponding parent compound.

Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

O-073

In Vitro Characterization and Structure-Activity Relationships of Methcathinone Analogues: Insights Into Stimulant Activity and Potential Risks

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Abstract

Introduction: Synthetic cathinones are one of the largest classes of New Psychoactive Substances (NPS) and have become increasingly prevalent in Europe in recent years. This is reflected by rising imports, seizures, and domestic production, particularly of methcathinone analogues, which accounted for 74% of NPS seizures in EU Member States in 2023. While 4-MMC (mephedrone) dominated the market in the late 2000s and early 2010s, compounds such as 3-MMC, 3-CMC, 4-CMC and 2-MMC have since emerged as replacements following control measures in several countries. This growing diversity of stimulant NPS poses significant health concerns and regulatory challenges, as structural modifications strongly alter potency and transporter selectivity, resulting in distinct pharmacological profiles. This highlights the need for their pharmacological characterization at the key mediators of their effects: the serotonin (SERT), dopamine (DAT), and norepinephrine (NET) transporters.

Objectives: This study aimed at comprehensively characterizing nine methcathinone analogues containing a methyl, bromo, or chloro substituent at the *ortho* (2), *meta* (3), or *para* (4) position of the aromatic ring (MMCs, BMCs, CMCs), and at elucidating structure-activity relationships (SARs) based on their transporter inhibition profiles.

Methods: Inhibitory potencies (IC_{50} values) at DAT, NET, and SERT were determined using the TRACT (transporter activity through receptor activation) assay in HEK293T cells, also enabling the calculation of DAT/SERT ratios as an indicator of abuse potential. In this assay, the extent of transporter-mediated neurotransmitter uptake inhibition is indirectly monitored *via* activation of the corresponding G protein-coupled receptor, resulting in a luminescent signal due to functional complementation of a split nanoluciferase enzyme. Furthermore, results were compared with those obtained using the fluorescence-based Neurotransmitter Transporter Uptake Assay (NTUA), in which transporter inhibition leads to a decrease in fluorescent signal.

Results: All compounds displayed a potency rank order of NET ≥ DAT ≥ SERT inhibition. In line with literature, activity at NET was similar across analogues, with 3-CMC being the most potent inhibitor (86.0 nM IC_{50}). At DAT, 3-BMC consistently exhibited the highest potency (95.7 nM IC_{50}), whereas the 2-substituted derivatives and 3-MMC were the least active (≥ 1051 nM IC_{50}). SERT inhibition was weak overall, with only micromolar potencies and especially low activity for the 2-halogenated derivatives (≥ 25,390 nM IC_{50}).

Discussion: Across all transporters, the 2-substituted analogues were the least potent inhibitors, indicating that *ortho* substitution is unfavourable for transporter inhibition, especially at SERT. Regarding DAT/SERT ratios, the 4-substituted derivatives comparatively showed the lowest ratios (ranging from 1.70-8.34), consistent with previous studies and their reduced reinforcing potential. In contrast, their 3-substituted counterparts consistently exhibited higher DAT/SERT ratios (between 9.41-18.7), suggesting greater abuse liability and stronger stimulatory

effects. Across all substitution positions, methylation resulted in lower DAT/SERT ratios than halogenation, in line with reports indicating that larger steric bulk is better tolerated at SERT than DAT. In terms of absolute potency, halogenation generally enhanced potency compared to methylation (Br > Cl > Me) at the 3- and 4-positions, while the opposite trend (Me > Cl > Br) was observed for the 2-substituted analogues. Overall, these findings provide crucial SAR insights into how both the nature and position of the ring substituent influence the transporter inhibition profiles of methcathinone analogues.

Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

O-074

Novel (Re)Emerging Substance? 5F-ADB Detection in Postmortem Toxicology

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Abstract

Introduction: 5F-ADB is a synthetic cannabinoid, a class of substances designed to mimic the effects of delta-9-tetrahydrocannabinol (THC), the primary psychoactive component of cannabis. Also known as 5F-MDMB-PINACA, it belongs to the indazole-3-carboxamide family and first emerged in Europe and the USA around 2014-2015. It acts as a potent full agonist at the cannabinoid receptors, producing more intense and less predictable effects than cannabis, including anxiety, tachycardia, hypertension, paranoia, hallucinations, and seizures. The substance was controlled by the US Federal government in 2016 and its prevalence in evidence seized by the Drug Enforcement Administration (DEA) peaked in 2018, but rapidly decreased over the next couple of years. Current DEA data shows a total number of 58 detections of 5F-ADB in 2022, down from a peak of 10,052 detections in 2018.

Objectives: The toxicology laboratory previously tested for 5F-ADB butanoic acid for several years until it was removed from the testing scope in 2022 due to a lack of positive results over the previous 3 years. In 2025, reports of the drug's re-emergence surfaced as it was again detected in the illicit drug supply and in postmortem toxicology casework in the USA. The toxicology laboratory revalidated the method and added the metabolite back to the scope of testing in October 2025.

Methods: At autopsy, blood specimens were collected in gray top tubes containing sodium fluoride and potassium oxalate as additives and sent to the laboratory. Samples received comprehensive testing to include volatiles analysis by headspace gas chromatography with flame ionization detection (GC-FID) and a screen for 350+ drugs of abuse, prescription drugs, and other agents by liquid chromatography with quadrupole time of flight mass spectrometry (LC-QToF-MS). Confirmation analysis included liquid chromatography with triple quadrupole mass spectrometry (LC-MS/MS) and LC-QToF-MS. As it is an unstable substance, 5F-ADB was confirmed as its 3,3-dimethylbutanoic acid metabolite via LC-QToF-MS with a 2 ng/mL reporting limit. The analytical method was validated following ASB guidelines to be qualitative.

Results: From October 1, 2025 to March 31, 2026, the lab detected 5F-ADB metabolite in 40 postmortem toxicology cases across 10 states – California (1), Florida (8), Indiana (4), Kentucky (4), Michigan (6), Missouri (1), Nebraska (6), Ohio (2), South Dakota (4), and West Virginia (4). Other substances detected in these cases include caffeine (20), naloxone (18), cotinine (12), MDMB-4en-PINACA butanoic acid (10), ethanol (4), and methamphetamine (4). 5F-ADB metabolite was detected as the only substance in 1 case. Excluding caffeine, cotinine, naloxone, and amiodarone, the metabolite was detected as the sole substance in 12 cases. Sixteen of the 40 decedents were inmates in prisons or correctional facilities.

Discussion: It is prudent to remember that while most novel substances emerge and then disappear rapidly from the illicit market, some do persist for months and years. But rarely, a substance will disappear from the market and then re-emerge years later as a drug of interest. 5F-ADB was scheduled by the government and its prevalence waned for years, but it re-emerged in 2025 and still maintains a presence as a drug of toxicological relevance in postmortem toxicology casework into 2026.

Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

O-075

From Ionic Homeostasis Modulation to Oxidative Stress: Toxicity Pathways of 4th-Generation Synthetic Cathinones

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Abstract

Introduction: The rapid emergence of New Psychoactive Substances (NPS) represents a major challenge for public health and forensic toxicology. Among these, synthetic cathinones (SCs) are one of the most prevalent classes, often featuring minor structural modifications that can still significantly affect their pharmacokinetic and pharmacodynamic properties. Despite their widespread use, their cellular and molecular mechanisms remain not fully understood. Available data suggests that para-halogenated derivatives of methcathinone may exhibit increased potency compared to non-substituted analogues such as methylmethcathinone (MMC). Therefore, further investigation into their cellular mechanisms is warranted to better elucidate their biological effects and potential risks to human health.

Objectives: This study aimed to compare the neurotoxicological profiles of two fourth-generation synthetic cathinones, 3-methylmethcathinone (3-MMC) and 3-chloromethcathinone (3-CMC), and to investigate how minor structural differences between these compounds may translate into distinct effects on neuronal cells.

Methods: An *in vitro* model based on human neuroblastoma cells differentiated into neuron-like cells with retinoic acid was used. For 3-MMC, cell viability was assessed using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay by testing increasing concentrations of the compound (10-300 nM) to determine sub-toxic conditions. Intracellular calcium homeostasis was evaluated using Fura-2AM video imaging. Current- and patch-clamp electrophysiology were performed to study membrane potential and ionic currents changes. Western blot analysis was used to study endoplasmic reticulum (ER) condition through ER stress and apoptotic markers. For 3-CMC, cytotoxicity was evaluated using MTT and LDH assays. Mitochondrial reactive oxygen species (ROS) production was analyzed by confocal microscopy using MitoTracker reagent.

Results: For 3-MMC, 300 nM was selected as the highest concentration that did not produce a significant reduction in cell viability (~95% viability), thus being considered sub-toxic. Although 300 nM did not significantly affect cell viability, it induced marked functional alterations. Results obtained demonstrate that it increased basal intracellular calcium levels and caused membrane depolarization. Electrophysiological recordings revealed increased voltage-dependent sodium currents and decreased potassium currents, leading to enhanced neuronal excitability. A reduction in the reverse activity of the Na^+/Ca^{2+} exchanger was also observed. Western blot analysis showed increased expression of Binding immunoglobulin protein (BiP) and Caspase-9, indicating ER stress and activation of apoptotic pathways. In contrast, 3-CMC showed clear cytotoxic effects in a dose-dependent manner. At 300 nM, a reduction in cell viability was already detectable, which became more significant at 10 µM. The LDH assay further confirmed these findings, showing

increased enzyme release consistent with loss of membrane integrity. In addition, confocal imaging demonstrated a marked increase in mitochondrial ROS levels, with fluorescence intensity increasing by approximately 71% compared to control cells, indicating a strong induction of oxidative stress and mitochondrial dysfunction.

Discussion: The results demonstrate that 3-MMC and 3-CMC exhibit distinct toxicological profiles despite their structural similarity. 3-MMC, at 300 nM concentrations, primarily alters neuronal, function by inducing hyperexcitability and disrupting calcium homeostasis without immediate cytotoxicity. In contrast, 3-CMC at 10μM exerts a predominantly cytotoxic effect, mediated by oxidative stress and mitochondrial dysfunction. These findings highlight the critical role of minor structural modifications in determining the biological activity of synthetic cathinones and provide insight into their potential mechanisms of toxicity, supporting future research aimed at identifying targeted therapeutic strategies.

Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

O-076

Development and Validation of a Sensitive LCMS^3 method for the Quantitative Analysis of Ethyl Glucuronide in Hair

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Abstract

Introduction: Ethyl glucuronide (EtG) is a metabolite of ethanol formed by UDP-glucuronosyltransferase-catalyzed glucuronidation of ethanol. It is used as a biomarker of ethanol consumption in various biological matrices, including hair. The "2019 Consensus for the use of alcohol markers in hair for supporting the assessment of abstinence and chronic alcohol consumption" published by the Society of Hair Testing states that hair EtG concentrations greater than or equal to 30 pg/mg strongly suggest excessive alcohol consumption, that concentrations greater than 5 pg/mg strongly suggest repeated alcohol consumption, and that concentrations lower than or equal to 5 pg/mg do not contradict self-reported abstinence. They define chronic excessive alcohol drinking as average daily consumption of 60 g or more of pure ethanol over several months. We herein describe a validated method for the extraction, purification, and analysis of EtG in hair by LCMS^3.

Objectives: To develop an LCMS^3 assay that can detect and quantify EtG in the relevant analytical range defined by the Society of Hair Testing while using a minimal amount of hair.

Methods: A 10 mg hair specimen is washed with isopropanol for 30 minutes in a culture tube in an oscillating water bath for 30 minutes at 37°C. The wash solution is removed. Internal standard (1000 pg of EtG-D5 in 0.050 mL methanol) and 1.950 mL of deionized water are added to the sample and it is heated at 100 °C for one hour to extract the EtG from the hair. The extract is solid-phase extracted using a 200 mg UCT ETG CARBON cartridge. The purified extract is reconstituted in 0.100 mL of deionized water. Analysis is performed on a Sciex 6500+ QTRAP in negative mode with Shimadzu LC30-AD binary pumps and a PAL HTC-xt autosampler with dynamic load and wash. The method utilizes a Waters Atlantis Premier BEH C18 AX 2.5 µm 2.1x100 mm column with mobile phases 0.1% acetic acid in water (A) and acetonitrile (B). The quantification mass transition is m/z 221→203→85. The qualifier is m/z 221→203→128. The internal standard utilizes m/z 226→208→85. The method employs a five-point quadratic curve with 1/x weighting.

Results: The linear range is 1 pg/mg to 150 pg/mg. The bias was assessed at 1, 2, 12, 30, 60, 100, and 150 pg/mg with less than 10% bias at all points. The between-batch precision tests at 15, 30, and 45 pg/mg yielded CVs less than 10%. The within-batch precision at 3, 15, 22.5, 30, 37.5, 45, 60, 120 pg/mg also yielded CVs less than 10%. The method was assessed for interferences, ion suppression, and dilution integrity with good results.

Discussion: This method provides the sensitivity described in other published methods, but it achieves it with a 10 mg sample while other published methods require 25-30 mg of hair. To the authors' knowledge, this is the first reported method to employ LCMS^3 technology for this application.

Disclosure

Yes, I, or a member of my immediate family, has a financial interest to disclose.

Conflict of Interest

I am a full-time employee and stock holder of Psychemedics Corporation, who markets this test.

O-077

LC-MS/MS Analysis of Drugs of Abuse in Human Nail Specimens: A Forensic Study of Drug Users in Jordan

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Abstract

Introduction: The detection of drugs of abuse is essential for understanding patterns of substance use and supporting forensic and public health investigations. Conventional biological matrices such as blood and urine provide relatively short detection windows, limiting their usefulness for retrospective drug exposure assessment. Human nails represent an alternative keratinized matrix capable of incorporating drugs and their metabolites over extended periods, offering a detection window ranging from several months to years. In addition, nail collection is non-invasive, simple, and resistant to adulteration. Liquid chromatography–tandem mass spectrometry (LC-MS/MS) provides high sensitivity and selectivity for the analysis of trace-level compounds in complex biological matrices and is therefore well suited for nail toxicology applications.

Objectives: This study aimed to develop and validate an LC-MS/MS method for the identification and quantification of five commonly encountered drugs of abuse—amphetamine, methamphetamine, Δ9-tetrahydrocannabinol (THC-COOH), cocaine, and alprazolam—in human nail specimens collected from Jordanian drug users. The study also sought to generate preliminary epidemiological data regarding drug-use patterns within the examined population.

Methods: Nail specimens were collected from 33 individuals with documented histories of drug abuse following approval and authorization from the Forensic Laboratory Directorate and the Anti-Narcotics Department of the Public Security Directorate of Jordan. Samples were decontaminated, pulverized, and subjected to extraction using validated laboratory procedures. Quantitative analysis was performed using a Shimadzu Nexera UPLC system coupled to an LCMS-8030 triple quadrupole mass spectrometer operating in multiple reaction monitoring (MRM) mode.

Method validation was conducted according to forensic toxicology guidelines and included assessment of selectivity, linearity, limits of detection (LOD), limits of quantification (LOQ), precision, and accuracy. Calibration standards were prepared across the validated analytical range.

Results: The developed LC-MS/MS method demonstrated satisfactory analytical performance, with coefficients of determination (R^2) ranging from 0.992 to 0.999. Limits of detection ranged from 3.016 to 8.18 pg/mg, while limits of quantification ranged from 9.14 to 24.79 pg/mg. Intra- and inter-day precision values showed relative standard deviations (RSDs) between 5.14% and 17.1%, and accuracy ranged from 95.2% to 99.4%.

Among the analyzed specimens, amphetamine was detected in 29 of 33 samples (87.9%), with concentrations ranging from 0.075 to 45.33 ng/mg. Methamphetamine was detected in 22 samples (66.7%), with concentrations ranging from 0.359 to 11.8 ng/mg. THC-COOH was identified in 4 samples (12.1%) at concentrations between 0.094 and 0.78 ng/mg. Cocaine was detected in

one sample (3.0%) at 2.44 ng/mg, while alprazolam was identified in three samples (9.1%) with concentrations ranging from 0.25 to 2.01 ng/mg.

Discussion: This study demonstrates the applicability of LC-MS/MS for the identification and quantification of drugs of abuse in human nail specimens, providing a sensitive and selective approach for long-term drug exposure assessment. The high prevalence of amphetamine and methamphetamine observed in the study population highlights the predominance of stimulant abuse among Jordanian drug users. Nail analysis represents a valuable complementary matrix for forensic toxicology investigations, retrospective drug-use monitoring, and epidemiological studies, particularly in cases where conventional biological specimens are unavailable or unsuitable.

Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

O-078

Pharmaco-Toxicological Discussion on the Incorporation in Hair of Four Doping Agents Consumed at an Identical Dose

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Abstract

Introduction: Hair analysis is widely used and well-established in forensic laboratories to document long-term exposure. However, interpretation of results remains challenging, particularly when comparing concentrations between compounds, even under similar exposure conditions.

Objectives: To analyze a hair specimen collected from a subject who used four anabolic agents and to explain the differences in concentrations observed despite identical dosing and administration time patterns.

Methods: A man, a non-professional athlete not participating in any competition, was involved in a doping case (drug transfer during intimate moments). The substances, prohibited by the World Anti-Doping Agency due to their anabolic effects, were consumed as part of a commercial mixture marketed under the name Muscle Building Stack (Biaxol), including ligandrol, ostarine, ibutamoren, and testolone, each labeled as 10 mg. Analysis by LC–MS/MS following methanolic extraction of crushed capsules revealed concentrations ranging from 8.8 to 10.4 mg per capsule. Quantification was performed using certified reference standards. The man reported taking one capsule per day of each substance over approximately four months.

A strand of hair (6 cm, black, oriented) was collected, segmented (6 × 1 cm), and analyzed using a published and validated method (Gheddar et al., J Chrom B, 2021, 15:1187:123048) by LC-MS/MS on a Waters XEVO TQSμ system.

Results: The method showed linearity over the range 0.5–1000 pg/mg. Limits of quantification were 1 pg/mg (ligandrol), 0.5 pg/mg (ostarine), 5 pg/mg (testolone), and 10 pg/mg (ibutamoren). Measured concentrations ranged from <1–4 pg/mg (ligandrol), 8–24 pg/mg (ostarine), 27–77 pg/mg (testolone), and 34–822 pg/mg (ibutamoren).

Discussion: Literature data report concentrations ranging from 1 to 65 pg/mg for ligandrol, 1 to 1105 pg/mg for ostarine, and around 224 pg/mg for ibutamoren. No data are currently available for testolone. In the light of these literature data, the concentrations observed are consistent with repeated use over several months. Moreover, the detection of the four molecules across all six segments supports this interpretation. Despite quite similar dosing and duration, marked difference in concentrations was observed between compounds. This variability likely reflects differences in incorporation mechanisms driven by physicochemical properties such as chemical structure, basicity (pKa), lipophilicity (LogP), and solubility. Notably, the number of nitrogen atoms increases from ligandrol (2) to testolone (5), potentially enhancing interaction with melanin and influencing accumulation. Lipophilicity also differs (LogP 0.7 for ibutamoren vs 3.6 for ligandrol), while testolone shows greater aqueous solubility than ostarine. These properties affect both incorporation and retention into hair, with less strongly retained compounds being more

susceptible to washout. Additional factors may include differences in extraction efficiency from the hair matrix and unknown stability after incorporation. Furthermore, possible enterohepatic cycling, particularly for testolone, may prolong systemic exposure. Overall, these findings demonstrate that, even under similar administration conditions, significant differences in hair concentrations can occur.

This study reports, for the first time, the detection of testolone in hair and highlights the importance of considering compound-specific properties when interpreting multi-drug exposure in hair analysis.

Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

O-079

Can the Macujo Method Evade Drug Detection in Hair? An Experimental Evaluation on Positive Samples

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Abstract

Introduction: Individuals required to undergo hair testing may seek methods to conceal drug use. The “Macujo method” is described on the internet as effective for that. The website offers persuasive arguments, phone support and well-structured tutorials.

Objectives: This study aimed to assess the efficacy of this method on hair samples previously tested positive in our forensic laboratory.

Methods: Four samples were included in the study. One sample, initially positive for ethyl glucuronide, was reanalyzed after 2 washing cycles, while 3 samples, initially positive for THC/cocaine/amphetamine/ketamine underwent 5 cycles.

The first step of the Macujo method involves washing with “Macujo Aloe Rid” (a detox shampoo). After rinsing, the hair is rubbed with baking soda for 5 to 7 minutes, then rinsed and dried again. A “Clean & Clear” lotion containing salicylic acid is subsequently applied for 30 minutes, followed by washing with liquid laundry detergent and again with Macujo reagent. The samples are then treated with apple cider vinegar before another layer of salicylic acid for 30 minutes, and another wash with laundry detergent. This cycle may be repeated for heavy users. The protocol recommends a final wash using “Zydot Ultra Clean”, an “internal hair purifying treatment” consisting of a three-step shampoo process.

All reagents were purchased in supermarkets or online. As the original Macujo reagent was not available outside the US, an alternative shampoo was selected using ChatGPT, to mimic real-world conditions.

Ethyl glucuronide was determined by LC-MS/MS after methanol extraction. The methods used for drugs of abuse analysis are accredited and routinely used. THC was analyzed by liquid-liquid extraction followed by gas chromatography while cocaine, amphetamine and ketamine were determined by LC-MS/MS after solid-phase extraction.

Results:

Ethyl glucuronide	**Before (pg/mg)**	**After (pg/mg)**	**Decrease (%)**
Sample 1	76	50	34.2
THC	**Before (ng/mg)**	**After (ng/mg)**	**Decrease (%)**
Sample 2	0.160	*0.0446*	70.9
Sample 3	0.259	0.175	32.4
Cocaine/Benzoylecgonine	**Before (ng/mg)**	**After (ng/mg)**	**Decrease (%)**
Sample 2	0.668 / 1.97	*0.490* / *1.74*	26.6/11.7
Sample 3	> 15 / 13.3	13.7 / 5.21	>8.7/60.8
Sample 4 segment 1 (proximal)	5.18 / 2.60	2.48 / 4.03	52.1/-55.0
Sample 4 segment 2	8.66 / 4.73	2.04 / 3.09	76.4/34.7
Sample 4 segment 3	9.48 / 5.82	1.73 / 2.48	81.8/57.4
Sample 4 segment 4	11.0 / 7.22	1.34 / 1.73	87.8/76.0
Amphetamine	**Before (ng/mg)**	**After (ng/mg)**	**Decrease (%)**
Sample 3	> 15	6.87	>54.2
Ketamine	**Before (ng/mg)**	**After (ng/mg)**	**Decrease (%)**
Sample 3	0.889	*0.196*	78.0

Discussion: Among the tested samples, only sample 2 (exhibiting the lowest initial concentrations) fell below the SOHT threshold. Other samples remained positive, although significant decreases were observed. This method, which was undetectable by visual inspection compared to dyeing or bleaching, proves effective at removing drugs from hair. Although even five cycles are already time-consuming, a greater number of cycles could potentially enable all samples to fall below the SOHT cutoff value. However, skin irritation and hair damage can not be excluded. Although the number of samples is too limited to draw definitive conclusions, the Macujo method appears to be effective in concealing low concentrations in hair. As toxicologists, the widespread use of this method may be a cause for concern.

Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

O-080

The Power of Chiral Amphetamine Analysis in Hair: Telling Therapy Compliance From Illicit Amphetamine Use

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Abstract

Introduction: Pharmacological treatment of attention-deficit/hyperactivity disorder (ADHD) with amphetamine-based products introduced the need in forensic toxicology to discriminate between the therapeutic use of medical products and the recreational use of illicit amphetamine. Medical amphetamine-based products are mostly enantiopure, whereas amphetamine on the illicit drug market comprises a racemic mixture. Therefore, chiral analysis of amphetamine (AMP) enantiomers enables the individual detection of (*S*)-AMP as a constituent of both medical products and illicit drugs, and (*R*)-AMP as a marker for illicit amphetamine use. By analyzing the hair matrix, the pattern of amphetamine use can be evaluated retrospectively over a period of several months. However, as hair analysis indicates the drug use *'on average'* over this period of time, interpretative challenges arise when different medication intakes and/or illicit drug consumption events contribute to the hair analysis result.

Objectives: In this retrospective study, the performance of chiral amphetamine analysis in hair was assessed in authentic casework from routine analyses at the Center for Forensic Hair Analytics (2017-2025, one segment n=299, two segments n=41). The power to discriminate between the use of medical products and illicit amphetamine was assessed, aiming at further resolving the interpretation of amphetamine-positive hair samples based on chiral analysis results.

Methods: For chiral amphetamine analysis, two liquid chromatography tandem mass spectrometry (LC-MS/MS) based methods have been used consecutively: An *indirect* method, detecting the diastereomers (*S*/*S*)- and (*R*/*S*)-AMP-(*N*-(2,4-dinitro-5-fluorophenyl)-L-valinamide) after chiral derivatization of amphetamine, and a *direct* method, separating the enantiomers (*S*)- and (*R*)-AMP directly with a chiral stationary phase. For the retrospective analysis, cases were anonymized, and ethical clarification was obtained. The chiral analysis results were expressed as enantiomeric fractions (EF) of (*S*)-AMP to the total amount of amphetamine in the sample (peak area of both enantiomers combined). Segmental hair analysis exemplified the challenging interpretation of combined therapeutic and illicit amphetamine use.

Results: Based on the analysis of quality controls and a comparison between chiral methods, a decision model was established. This model was used to categorize hair samples as 'in accordance with therapy compliance' ($EF \geq 0.95$, n=284), 'in accordance with illicit amphetamine use' ($EF \leq 0.55$, n=32), and 'indicative of combined use of medical and illicit amphetamine' ($0.55 < EF < 0.95$, n=24) within the overall investigated time window. Importantly, out of 264 cases showing amphetamine concentrations in hair above the recommended reporting cut-off (200 pg/mg), 222 cases showed an $EF \geq 0.95$. Segmental hair analysis revealed different trends, related to different interpretations of combined use, based on (the lack of) longitudinal differences in the EF.

Discussion: The enantioselective detection of amphetamine plays a crucial role in avoiding false positives regarding illicit amphetamine use. The proposed decision model allows to evaluate the nature of amphetamine use based on the (*S*)-AMP EF. Notwithstanding, an EF between 0.55-0.95 can only be categorized as 'indicative' as multiple scenarios can explain such results: therapy with a specific product (Adderall®, i.e., mixed amphetamine salts), illicit amphetamine side consumption during medical therapy, temporal shifts in consumption pattern, and/or hair outgrowth. Preliminary insights show, however, that segmental analysis could further narrow down the origin of amphetamine intake.

Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

O-081

Uncovering Drug Use at the Time of a Criminal Offense: Segmental Hair Analysis in a Forensic Psychiatric Population

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Abstract

Introduction: The present study is led by the Swedish National Board of Forensic Medicine, in which hair samples were collected from individuals undergoing forensic psychiatric evaluation. Hair analysis is established in forensic toxicology. Since it presents good stability of analytes within the hair matrix, it offers a long detection window, allowing for a retrospective evaluation of substance use.

Objectives: Evaluate adherence to prescribed medications and to identify potential use of illicit substances at the time of a criminal offense through segmental hair analysis. Method validation was performed following ANSI/ASB Standard 036, First Edition 2019 "Standard Practices for Method Validation in Forensic Toxicology" and the Society of Hair Testing guidelines for hair analysis.

Methods: Hair segments (10 mg; 1 cm) were washed at 37°C with isopropanol (15 min), then three times with phosphate buffer (pH6.0, 30 minutes, each), with a final rinse step with isopropanol. After drying, samples were pulverized on Biotage Lysera (6 min, speed of 5.30, 20°C). Samples were then centrifuged (15000 RPM, 20 min, 20°C) and transferred to filter vials and 10 µL was injected into an Agilent 6550 iFunnel QTOF-MS for analysis.

Results: The method demonstrated selectivity for 60 target analyte-s (13 opiates, 6 stimulants, 9 antidepressants, 8 benzodiazepines and Z-drugs, 19 antipsychotics, and 5 sedatives/antihistamines), with working ranges between 50 and 8000 pg/mg. Depending on the analyte, lower limits of quantification (LLOQs) of 100 or 200 pg/mg and upper limits (ULOQ) of 4000 pg/mg were also established. Inter-day and intra-day precision (2-13%) and accuracy (bias within ±20%) were met for all analytes. Significant ion enhancement (>25%) was observed for 6-acetylmorphine, 7-aminoclonazepam, benzoylecgonine and oxycodone, while ion suppression was noted for dehydroaripiprazole, however, these matrix effects did not impact their LLOQs. No carryover was observed. Application to authentic samples confirmed the presence of several analytes within the method working range (7-aminoclonazepam, 7-aminonitrazepam, 7-OH-quetiapine, 9-OH-risperidone, 6-acetylmorphine, alimemazine, alprazolam, amphetamine, aripiprazole, benzoylecgonine, citalopram, clozapine, cocaethylene, cocaine, codeine, desmethylalimemazine, diazepam, flupentixol, haloperidol, hydroxyzine, levomepromazine, MDMA, methadone, mirtazapine, morphine, norclozapine, nortriptyline, olanzapine, O-desmethyltramadol, promethazine, propiomazine, quetiapine, risperidone,

sertraline, tramadol, venlafaxine, ziprasidone, zolpidem, zopiclone, and zuclopenthixol). A case example is shown in Figure 1 where hair segment S2 corresponds to the time of the offense.

Medication	Haloperidol (until 2021-11-25)
Illicit drugs	Alcohol, Heroin, Cocaine
Hair colour	Brown
Date of sampling	15/12/2021
Date of event	26/10/2021

	Nov 2021	**Oct 2021**	**Sep 2021**	**Aug 2021**	**July 2021**
Analyte concentration (ng/mg)	**S1 (1cm)**	**S2 (1cm)**	**S3 (1cm)**	**S4 (1cm)**	**S5 (1cm)**
Haloperidol	1.70	1.00			
Zuclopentixol	0.10	0.10			
Codeine			0.17	0.24	0.332
Morphine			0.81	1.10	1.50
6-acetylmorphine	0.14	0.31	1.80	3.60	5.50
Cocaine	0.18	0.83	5.30	9.50	12.20
Cocaine; Benzoylecgonine	0.12	0.46	2.80	5.90	69.70
Cocaine; Cocaethylene		0.10	0.56	0.57	0.53

Figure 1 – Segmental hair analysis showing concentrations (ng/mg) of antipsychotic medication and illicit drugs across sequential time periods.

Discussion: Our method fulfilled the validation criteria, enabling the detection and quantification of 60 substances in human hair. Moreover, sample preparation was straightforward, no further extraction step was required making it suitable for high throughput in forensic toxicology applications. The case example illustrates how segmental hair analysis can reveal changes in drug use and medication over time. One interpretation can be that non-compliance of haloperidol medication in combination with use of cocaine and heroin may have triggered the violent crime. After apprehension, medication with haloperidol is re-established, zuclopenthixol initiated, and drug use ceases even though residual drugs are seen in proximal hair segments.

In conclusion, the method was found suitable for the quantitative determination of a range of relevant drugs and medications in hair to explore the temporal pattern of use.

Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

O-082

Orbitrap HRMS-Based Workflow for Hair Analysis to Reveal Hidden Patterns of NPS Exposure

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Abstract

Introduction: In forensic toxicology, biological matrices such as blood and urine are routinely employed to assess recent drug intake. Hair analysis offers a complementary yet underutilized perspective in the investigation of NPS. Due to its incorporation into the keratin matrix, hair can retain molecules over extended periods, enabling retrospective assessment of drug exposure. In addition, hair is less affected by short-term abstinence or adulteration and supports segmental analysis for temporal interpretation. Despite these advantages, systematic screening procedures for NPS in hair remain limited. The analytical challenge lies in detecting a wide range of structurally diverse compounds, often in the absence of reference materials. Furthermore, established techniques such as GC–MS and LC-MS/MS provide robust analytical performance but may lack the flexibility required for the detection of emerging NPS.

Objectives: This study aimed to develop and apply an untargeted HRMS workflow for the identification of NPS in hair samples, enhancing both analytical capability and interpretative value in forensic casework.

Methods: An untargeted workflow based on LC–HRMS with Orbitrap was applied to authentic hair samples. Approximately 20 mg of hair were decontaminated, pulverized, and subjected to overnight extraction in methanol after incubation at 55 °C for 2 hours. Chromatographic separation was achieved using a Kinetex core–shell C18 column (1.7 µm, 100 × 2.1 mm) with a total run time of 16 minutes. Data were acquired in full-scan mode with data-dependent MS/MS fragmentation (HCD) using ESI+ ionization.

Data processing was performed using Compound Discoverer. Compound identification was supported by accurate mass (≤5 ppm), isotopic pattern, and MS/MS fragmentation consistency, achieving Level 2 confidence according to published identification criteria. Compound annotations were assigned through spectral matching against libraries (HighResNPS, mzCloud) integrated within the Compound Discoverer workflow. In a typical toxicological sample, the filtering strategy is able to reduce over 30,000 initial annotations to 10–20 relevant ones. Conventional selectivity was assessed by multiple injections of 6 pooled negative samples. A novel approach to assess Library Match selectivity was also developed. The Limit of Identification (LOI) was determined after triplicate injections of spiked hair with 203 reference compounds at 25, 50 and 100 ppb.

Results: Overall, 70.9% of the 203 spiked compounds were identified at 25 ppb, increasing to approximately 80% at 50 ppb, proving adequate sensitivity of the method. No false-positive identifications were observed, although two probable annotations to flunitazene were reported among the six negative samples. Application to authentic cases enabled the identification of multiple NPS, including MDMB-4en-PINACA and the synthetic cathinone MDPHP, revealing patterns of polydrug use and repeated exposure. The untargeted approach also allowed the detection of further compounds (e.g. metabolites) which can assist the interpretation of the origin of the exposure.

Discussion: These findings are consistent with international reports describing intoxication cases related to synthetic cathinones and cannabinoids across Europe. Beyond case-specific interpretation, the proposed workflow may support early detection of emerging NPS and improve epidemiological surveillance where conventional monitoring systems remain limited. Future integration with molecular networking approaches could facilitate the recognition of structurally related compounds, metabolites, and transformation products, thereby supporting public health authorities and stakeholders in responding to the evolving drug landscape.

Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

O-083

Optimization and Validation of a Method for Detecting Single Dose Drug Exposure in Hair: Application to Authentic Specimens

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Abstract

Introduction: Hair is a valuable matrix in forensic toxicology due to its extended detection window. Drugs take ~10 days to incorporate into the hair shaft and emerge from the scalp, after which they can remain detectable for weeks to months after ingestion. This can be especially helpful in cases of drug-facilitated sexual assault (DFSA) and drug-facilitated crime (DFC), where exposure often involves a single administration. Despite this advantage, hair analysis remains analytically challenging, involving numerous preparatory steps and variable experimental conditions.

Objectives: The primary aims of this study were to optimize a hair extraction procedure and to develop a LC-QqQ-MS method capable of detecting drugs at concentrations consistent with single-dose exposure. A Design of Experiments (DoE) framework was employed for additional optimization of hair extraction parameters for drugs and metabolites relevant to DFSA/DFC using authentic hair reference materials (HRM). An LC-QqQ-MS analytical method was validated in accordance with the ANSI/ASB Standard 036, with particular emphasis on low limits of detection (LOD) and quantification (LOQ) for single dose detection.

Methods: Hair samples were decontaminated and homogenized into a powder following established protocols. Extraction was performed using a solvent-swelling technique. Hair samples were incubated at 37°C in methanol:acetonitrile:2 mM aqueous ammonium formate (25:25:50). A 2^3 full factorial DoE design evaluated the effects of three parameters: sample mass (5 vs. 20 mg), extraction duration (2 vs. 6 h), and ultrasonication (used vs. not used). Quantitative analysis was conducted using an Agilent 1290/6470 LC-QqQ-MS, with quantification of 40 drugs and metabolites. Authentic single dose specimens were obtained and analyzed to assess method performance. Samples from an IRB approved single dosing study involving 30 individuals given 25 mg diphenhydramine (DPH) followed by hair collection at 30/60 days after dosing. In addition, two authentic single dose specimens suspected to contain cetirizine, sertraline and lisdexamphetamine were also analyzed. All samples were processed for segmental analysis, where hair was cut into 5 mm segments from root to tip and analyzed independently by the validated method.

Results: DoE optimization demonstrated that a 5 mg HRM sample and a 6-h extraction time without ultrasonication yielded the highest recoveries for multiple drugs. The validated LC-QqQ-MS method exhibited excellent selectivity, precision, and accuracy, achieving low LOD/LOQ values suitable for detecting trace-level drug incorporation. DPH was detected in all specimens at both 30 and 60 days post dosing at varying concentrations. Numerous specimens exhibited a classical profile characterized by a DPH peak closer to the scalp in 30-day as compared to 60-day specimens, although segments with the highest DPH levels differed among individuals. Furthermore, several subjects exhibited more complex patterns of DPH incorporation (*e.g.*, broad profile or multiple peaks). The two additional single dose specimens were found to contain cetirizine (in both) and sertraline (in one), with no detection of lisdexamphetamine.

Discussion: This study presents a fully optimized and validated method for quantitative drug analysis in hair capable of detecting levels of drugs relevant to single dose exposure. The LC-QqQ-MS procedure demonstrated excellent performance for all 40 analytes, with applicability tested using known single dose specimens. Analysis of authentic single dose specimens with additional drugs is currently underway.

Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

O-084

Development and Validation of a Homogeneous Enzyme Immunoassay Screen for PCP Analysis in Hair

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Abstract

Introduction: PCP is a dissociative hallucinogen with no legitimate medical use, and is part of the Federal panel of tested drugs. Herein, we describe development and validation of a PCP homogenous enzyme immunoassay screen for hair. The development process consisted of synthesis of a carboxylic acid modified PCP-like antigen from commercial starting materials. The antigen was conjugated to high-activity glucose-6-phosphate dehydrogenase (G6PDH) via a specific coupling and purification sequence. The resulting PCP-G6PDH conjugate was screened against commercially available monoclonal antibodies to develop a homogeneous immunoassay at a cutoff of 300 pg PCP/mg hair.

Objectives: To develop a homogeneous enzyme immunoassay screen to detect 300 pg PCP/mg hair using a custom PCP-G6PDH conjugate paired with a commercially available monoclonal antibody.

Methods: Synthesis of a carboxylic acid modified PCP antigen was performed in four steps. The carboxylic acid modified antigen was conjugated via amide bond coupling to G6PDH using a procedure optimized for enzyme deactivation, as well as antibody-induced enzyme inhibition. Commercially available mouse monoclonal antibodies were screened for optimized inhibition against the PCP-G6PDH conjugate via an AU 640 analyzer at a cutoff of 300 pg PCP/mg hair. Hair samples were screened following drug extraction using phosphate buffer heated to 75° C. A comparison study was performed with a new hair aliquot that was first washed using an extended aqueous wash, followed by enzymatic hair digestion, liquid-liquid extraction and confirmation using an Agilent GC/MS.

Results: Cross-reactivity of the immunoassay measured against small molecule analytes yielded 100% cross-reactivity with venlafaxine and 3% cross-reactivity with atropine. Cross-reactivity of the immunoassay with structurally similar PCP analogs determined 30% cross-reactivity with 3-Methoxy-(aryl ring) PCP and 12% cross-reactivity with 4-Hydroxy-(cyclohexyl ring) PCP. Evaluation of potential negative and positive assay interferences against 110 individual small molecule analytes at a concentration of 10,000 pg interference/mg hair yielded venlafaxine, atropine and chlorpheniramine maleate as positive immunoassay interferences. Intra- and inter-assay precision was assessed at -50%, -25%, cutoff, +25%, +50%, +75% and +100% of the cutoff. Linearity was established between -50% and +100% of the cutoff. A comparison study of the immunoassay determined one sample was positive by immunoassay screen, but contained PCP below the cutoff after an extended aqueous wash.

Discussion: This work describes development of a methodology to rapidly screen for PCP in hair by homogenous immunoassay at a cutoff of 300 pg PCP/mg hair. The assay was developed by synthesis of a carboxylic acid modified PCP antigen, followed by antigen coupling to G6PDH via amide bond formation using glucose-6-phosphate as a necessary additive during the coupling process. The PCP-G6PDH conjugate could be inhibited by a commercial monoclonal antibody.

The homogeneous assay was put through an analytical validation including cross reactivity, interferences, precision and linearity. A GC/MS confirmed sample comparison study consisting of n = 94 samples found one hair sample that was immunoassay screen positive contained PCP above the cutoff prior to sample washing, but confirmed negative with PCP close to the calibrator after an extended aqueous wash.

Disclosure

Yes, I, or a member of my immediate family, has a financial interest to disclose.

Conflict of Interest

Neil Stowe, Elvan Loni, Ryo Furukawa, Alejandro Gonzalez and Ryan Paulsen are employees of Psychemedics. Neil Stowe and Ryan Paulsen are stockholders in Psychemedics.

O-085

Brain: Femoral Blood Ratios of Amphetamine, Benzoylecgonine, Cocaethylene, Cocaine, Methamphetamine, and Fentanyl

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Abstract

Introduction: Amphetamine (AMP), benzoylecgonine (BE), cocaethylene (CE), cocaine, methamphetamine (MAMP), and fentanyl remain prevalent analytes in postmortem toxicology casework. Brain (BR) has utility for postmortem toxicology testing because of its ample quantity, ease of collection and testing, and insusceptibility to putrefactive changes. Reference BR concentrations samples are limited; therefore, interpretation of these results can be challenging.

Objectives: To determine BR: femoral blood (BL) ratios of AMP, BE, CE, cocaine, MAMP, and fentanyl.

Methods: Cases involving homicide, decomposition, and/or head trauma were excluded. For fentanyl, hospital cases were excluded to eliminate therapeutic fentanyl administration. BL samples were collected in gray top tubes and refrigerated. BR samples were collected from the occipital lobe and frozen. BR samples were extracted in triplicate and BL samples were extracted once by solid phase extraction. Instrumental analyses were performed using liquid chromatography tandem mass spectrometry. The method was validated to determine BR concentrations using a BL-based calibration (AMP/MAMP range: 5-1000 ng/mL, BE/CE/cocaine range: 5-2000 ng/mL, fentanyl range 0.5-100 ng/mL). The limits of quantitation in BR were 1.5 ng/g for fentanyl and 5 ng/g for the stimulant drugs BR. Only cases with quantitative results for BR and BL were included.

Results: Cases included were as follows: AMP (n=17), BE (n=46), CE (n=17), cocaine (n=60), MAMP (n=20), and fentanyl (n=69). The ranges of BR: BL ratios were AMP (2.2-5.8), BE (0.2-2.1), CE (1.2-5.1), cocaine (0.1-5.8), MAMP (2.2-7.3), and fentanyl (1.1-24.8).

BR concentrations were always greater than BL concentrations for AMP, CE, MAMP and fentanyl. Similarly, BR concentrations were more abundant for most cocaine cases (95%). In contrast, few BE-positive cases had higher BR than BL concentrations (13%).

There was no significant difference in BE BL:BR ratios when cocaine was or was not present Correlation plots indicated a slight positive correlation between BL and BR concentrations for all analytes.

Discussion: Analytes demonstrating BR:BL ratios greater than 1 (AMP, CE, cocaine, MAMP, and fentanyl) are characterized by moderate to high lipophilicity and substantial volumes of distribution. Fentanyl exhibited the highest mean ratio and greatest variability, consistent with its high lipid solubility and known susceptibility to postmortem redistribution. Because femoral BL concentrations may not reflect perimortem concentrations, interpretating fentanyl BR:BL ratios are further complicated.

In contrast, BE displayed a mean BR:BL ratio below 1. As a polar metabolite, BE is less likely to accumulate in the lipid-rich BR. Additionally, hydrolysis of cocaine to BE may elevate BL BE concentrations, thereby lowering observed BR:BL ratios. The reported BE BR:BL ratios may be impacted by inclusion bias. Cases were excluded when a concentration was not obtained. This was most common when BL BE concentrations exceeded the upper limit of quantitation. It is possible that BR:BL ratios behave differently at higher BE concentrations.

These data support BR as a useful alternative matrix for postmortem toxicology and provides reference data for BR:BL ratios. Because of the variability in BR:BL ratios, BR concentrations should not be used as a direct proxy for BL concentrations.

Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

O-086

Simethicone-Associated Pharmacobezoar Formation in Fatal Multi-Drug Overdose: Clinical and Toxicological Insights

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Abstract

Introduction: Pharmacobezoars are rare intragastric aggregates of drugs that may significantly affect absorption kinetics and complicate toxicological interpretation. Their role in fatal intoxications following multi-drug ingestion remains insufficiently characterized in forensic toxicology.

Objectives: To present a fatal case of intentional multi-drug ingestion associated with pharmacobezoar formation and to evaluate its impact on postmortem toxicological findings and interpretation.

Methods: A fatal case involving a 21-year-old woman was investigated following intentional ingestion of multiple pharmaceuticals, including immediate- and extended-release metformin, simethicone, drotaverine, paracetamol, nifuroxazide, and desloratadine. Clinical data from a three-day intensive care hospitalization were reviewed. A full forensic autopsy was performed. Toxicological analyses of postmortem blood, urine, and intragastric pharmacobezoar material were conducted using validated ultra-high-performance liquid chromatography–tandem mass spectrometry (UHPLC-QqQ-MS/MS) method.

Results: On admission, the patient presented with severe lactic acidosis, hypoglycemia, and early renal impairment. Despite intensive treatment, progressive metabolic acidosis and multi-organ failure developed, leading to death on the third hospital day. At autopsy, a compact intragastric mass consistent with a pharmacobezoar was identified, with macroscopic features suggesting a simethicone-based aggregate. Toxicological analysis confirmed the presence of ingested drugs in postmortem blood, urine, and pharmacobezoar material. Notably, the coexistence of systemic drug concentrations and substantial amounts of retained intragastric material indicates incomplete gastrointestinal elimination and ongoing drug availability prior to death.

Discussion: Simethicone, although considered pharmacologically inert, may facilitate the aggregation of co-ingested solid medications due to its surface-active properties. In the presence of extended-release formulations and multiple orally administered drugs, this may promote pharmacobezoar formation, prolong intragastric retention, and sustain drug absorption. This case highlights a potentially underrecognized mechanism contributing to refractory toxicity in overdose. Pharmacobezoar formation should be considered in cases of persistent or worsening clinical status despite standard treatment. Early recognition may support targeted diagnostic and therapeutic interventions in acute toxicology.

Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

O-087

Is Red Bone Marrow a Reliable Matrix for Estimating Blood Ethanol in Decomposed Bodies? A Pilot Study with Focus on the Lipid Fraction

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Abstract

Introduction: Cadaver decomposition can severely compromise toxicological analyses in conventional matrices. Bone marrow (BM), located within the medullary cavity and protected by cortical bone, has emerged as a valuable alternative matrix when blood and soft tissues are unavailable or degraded. BM is classified into red (hematopoietic) and yellow (fatty) types, and their distribution varies by anatomical sites: rib and iliac crest marrow are predominantly hematopoietic, while femoral marrow is primarily fatty. BM lipids may influence xenobiotic distribution according to their physicochemical properties. Evaluating the BM lipid fraction and its changes during decomposition may be crucial for interpreting postmortem toxicological findings.

Objectives: This study aimed to: (i) develop and validate a rapid semi-quantitative spectrophotometric method to assess lipid content in red BM (RBM) and psoas muscle (PM); (ii) assess whether lipids affect ethanol concentrations in well-preserved (WP) and decomposed (D) bodies; (iii) explore predictive models to estimate femoral blood (FB) ethanol from RBM.

Methods: Lipids was measured in ribs RBM and PM from 63 cadavers (24 WP, 39 D) using a sulfo-phospho-vanillin (SPV) colorimetric assay specifically validated for postmortem matrices. Ethanol (LOQ 0.05 g/L) and ethyl glucuronide (EtG) (LOQ 2.9 ng/mL) were quantified by validated HS-GC-FID and LC-MS/MS methods in FB, RBM, and PM from 166 cadavers (76 WP, 90 D).

Results: Lipids were significantly higher in D cases compared to WP cases in both RBM (p=0.010) and PM (p=0.049). However, linear regression analysis showed no significant relationship between lipid and ethanol concentrations in both matrices. Mean and median ethanol ratios were, respectively, 0.99±0.36 and 0.97(FB/PM), 2.74±1.08 and 2.62 (FB/RBM), 2.84±1.18 and 2.77 (PM/RBM), without significant differences between WP and D. Significant linear regression models were found between RBM and both FB (b_1=2.3, p«0.001, R^2=0.79) and PM (b_1=1.97, p«0.001, R^2=0.81) ethanol concentrations. Moreover, in approximately 18% of D cases, ethanol was >LOQ in FB and/or PM, while remaining <LOQ in RBM. In all these cases, EtG was <LOQ.

Discussion: Despite significant increase in lipid content in D tissues, lipids was not related to ethanol concentrations in RBM or PM, contrary to the expected reduction of the available aqueous phase. The absence of an observable effect suggests that other factors may mitigate the impact of lipid fraction on ethanol distribution. The lack of variations in ethanol ratios across different preservation states, together with the strong relationship between RBM and FB ethanol, indicate that RBM – potentially even more reliably than PM – can provide a reliable estimation of FB ethanol. Finally, RBM analysis may assist in differentiating postmortem ethanol production and antemortem intake. D cases with ethanol >LOQ only in FB and/or RBM but EtG <LOQ suggest postmortem neoformation.

Conclusions

A semi-quantitative spectrophotometric method was applied to determine lipid content in RBM and PM, allowing us to investigate and ultimately exclude a relevant influence of lipids on ethanol concentrations in these matrices. Accordingly, linear (and possibly non-linear) regression between RBM and FB ethanol concentrations proved highly promising as a tool to estimate FB ethanol, particularly in decomposed bodies where conventional matrices are unavailable or unreliable.

Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

O-088

Contrasting Indicators: Aligning Overdose Mortality and Drug Seizure Trends in Arkansas

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Abstract

Introduction: Drug overdose mortality and illicit drug supply indicators represent interconnected aspects of the substance use landscape. In Arkansas, overdose deaths rose sharply during the COVID-19 pandemic, driven largely by synthetic opioids and stimulant use, particularly fentanyl and methamphetamine. From 2022 to 2024, deaths declined steadily, returning to 2019 levels. Despite this improvement, the illicit drug supply continues to evolve, presenting ongoing public health and forensic challenges. Overdose mortality data were obtained from the Arkansas State Crime Laboratory.

Law enforcement seizure data show statewide increases in controlled substance availability and trafficking. By 2025, Arkansas reported record-setting seizures, reflecting its role as a major transit corridor. The concurrent decline in overdose deaths and rise in seizures underscores the complex relationship between drug supply, harm-reduction efforts, and mortality. Seizure data were obtained from the Arkansas State Police (ASP) and Drug Task Force (DTF). Understanding how overdose trends align—or diverge—from seizure patterns is essential for public health surveillance, forensic toxicology, and policy response.

Objectives: To evaluate sequential trends in overdose mortality in Arkansas from 2021 to 2025, with emphasis on fentanyl and methamphetamine, and to compare these trends with law enforcement seizure data and harm-reduction initiatives.

Methods: This retrospective observational study integrated statewide overdose mortality data with ASP and DTF drug seizure records from 2021–2025. Overdose deaths were analyzed by year, drug class, and demographic characteristics when available. Drug seizure data were categorized by substance type and time frame. Temporal trends were assessed using year-to-year percent change and qualitative comparisons to evaluate alignment between shifts in drug supply and overdose mortality.

Results: Overdose deaths peaked in 2021 (495 deaths) before declining to 365 deaths in 2025. In 2025, 65% of deaths involved fentanyl or methamphetamine, and 9% involved both substances. Male decedents accounted for 63% of deaths. Individuals aged 35–99 represented 79% of overdose deaths, with methamphetamine detected in 53% of cases and opioids in 34%, including fentanyl in 61% of opioid-positive deaths.

Drug seizures increased substantially over the study period. In 2021, ASP and DTF seized approximately 545 kg of methamphetamine and 15 kg of fentanyl; by 2025, seizures rose to more than 1,400 kg of methamphetamine and 141 kg of fentanyl. Methamphetamine seizures nearly doubled between 2024 and 2025 and tripled since 2023. Fentanyl seizures fluctuated, with declines in 2024 followed by increases in 2025. Reporting gaps remain due to inconsistent data submission across more than 350 law enforcement agencies statewide.

Discussion: The divergence between declining overdose mortality and increasing drug seizures underscores the limitations of interpreting supply-based indicators in isolation. Expanded naloxone access, harm-reduction initiatives, shifts in drug use behaviors, and policy changes likely contributed to reduced mortality. However, polysubstance use—particularly fentanyl and methamphetamine—remains a major driver of overdose risk. Methamphetamine-involved deaths continue to rise despite increased seizure activity, highlighting the need for integrated forensic, public health, and law enforcement data systems to characterize Arkansas's evolving drug landscape. It remains unclear whether criminal organizations are creating demand, fueling economic opportunity, or merely supplying an existing market.

Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

O-089

Ongoing Decline in Fentanyl-Related Mortality in the United States: Evidence for Contribution From a Supply-Driven Inflection Within a Complex Public Health Response

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Abstract

Introduction: Fentanyl-related overdose mortality in the United States increased dramatically over the past decade, reaching a peak in 2023. Since mid-2023, national surveillance data indicate a sustained decline in overdose deaths. Postmortem forensic toxicology data provide a unique opportunity to examine changes in drug positivity, concentrations, and co-occurring analytes that may help explain this reversal and inform prioritization of ongoing public health interventions. Potential contributing factors include changes in the illicit fentanyl supply, expanded harm reduction and treatment access, and behavioral adaptation among people who use drugs.

Objectives: To use forensic toxicology data and current evidence from mortality and drug supply data to examine the potential causes of the recent deceleration in fentanyl-related mortality in the United States.

Methods: Qualitative and quantitative toxicology data were extracted from the NMS Labs laboratory information system for postmortem cases with blood analyzed by time-of-flight mass spectrometry between Q1 2018 and Q1 2026. Trends in fentanyl, naloxone, and methamphetamine positivity were evaluated over time using segmented regression with Akaike Information Criterion (AIC)–based breakpoint selection. Fentanyl blood concentrations were assessed using Spearman rank correlation. Co-occurrence of naloxone and methamphetamine was evaluated among fentanyl-positive cases. All statistical analyses were performed in R.

Results: Fentanyl positivity followed three primary phases: early escalation (Q1 2018–Q2 2021), late escalation/plateau (Q2 2021–Q2 2023), and post-peak bi-phasic decline (Q2 2023–Q1 2026). During the three-year early escalation period fentanyl positivity increased by approximately 1.3% per quarter, slowing to 0.15% per quarter during the plateau phase. Between mid-2023 and mid-2024 positivity declined rapidly at a rate of approximately –2.8% per quarter before slowing, returning to levels comparable to those observed at the beginning of the study period within approximately 1.5 years. Despite declining positivity, median fentanyl blood concentrations increased significantly over time. Naloxone co-occurrence among fentanyl-positive cases demonstrated a small but steady increase (approximately 0.04% per quarter) across the study period. Methamphetamine co-occurrence in fentanyl-positive changes were 1.6%, 0.80%, and 0.29% in the early escalation, late escalation/plateau, and post-peak decline phases, respectively.

Discussion: Multiple indicators suggest that the decline in fentanyl-related mortality beginning in mid-2023 is temporally associated with a disruption in the illicit fentanyl supply. The rapid decline in fentanyl positivity observed in forensic toxicology data closely parallels national mortality trends and aligns with external evidence of decreased fentanyl availability, reduced purity, and changes in upstream supply chains. This interpretation is strengthened by parallel trends observed in Canada. Further data supporting supply chain changes in decreased mortality include purity and availability of drug, as shown in data from the Center for Forensic

Science Research and Education. The steady increase in naloxone co-occurrence is consistent with expanded naloxone access. Together, these findings are consistent with a supply-driven negative inflection in fentanyl mortality and highlight the value of forensic toxicology data in contextualizing national overdose trends.

Disclosure

Yes, I, or a member of my immediate family, has a financial interest to disclose.

Conflict of Interest

Both authors are employees of NMS Labs, a commercial forensic toxicology reference laboratory

O-090

Evidence of Multifactorial Ketoacidosis in a Postmortem Toxicology Case: Resolving Discrepant Inter-Matrix Ethanol Findings Through Beta-Hydroxybutyrate Quantification and Case History

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Abstract

Introduction: Ethanol is among the most frequently detected analytes in forensic toxicology, and its postmortem interpretation carries substantial medicolegal implications. Supplementary matrices such as vitreous humor (VH) and urine are routinely tested to distinguish antemortem consumption from postmortem production. Interpretation is further complicated by endogenous ethanol formation, putrefactive change, and concurrent metabolic disease. Ketoacidosis — characterized by accumulation of acetoacetate, acetone, and beta-hydroxybutyrate (BHB) — arises from insulin deficiency, excessive alcohol use, or acute starvation, each altering the metabolic pathway that confounds standard alcohol analysis. BHB is the preferred ketoacidosis biomarker given the postmortem instability of glucose and non-specificity of acetone.

Objectives: This case report describes the investigation of discrepant ethanol concentrations across multiple biological matrices, illustrates the diagnostic utility of BHB testing and case history in resolving interpretive conflicts, and highlights a rare triple metabolic acidosis as a probable contributor to death in an insulin-noncompliant diabetic decedent.

Methods: Aortic blood, VH, and urine were collected at autopsy from a 45-year-old male with an approximately two-day postmortem interval. All specimens underwent a routine alcohol panel — ethanol, methanol, acetone, and isopropanol — via headspace gas chromatography with flame ionization detection. Aortic blood was screened for drugs by liquid chromatography–time-of-flight mass spectrometry (LC-TOF). Vitreous glucose was measured spectrophotometrically and BHB was quantified in blood by gas chromatography–mass spectrometry. VH and urine were reanalyzed at multiple time points over seven months to assess stability and endogenous production trends.

Results: Aortic blood yielded no detectable ethanol; acetone was elevated at 29–30 mg/dL. VH ethanol measured 256 mg/dL initially, declining to 118 mg/dL across subsequent analyses, with concurrent decreases in VH acetone (32 to 8.3 mg/dL) and rising isopropanol (13 to 43 mg/dL). Urine ethanol was markedly elevated and highly stable across three analyses spanning four months (437–440 mg/dL). Vitreous glucose was 676 mg/dL, consistent with profound antemortem hyperglycemia. BHB was significantly elevated at 920 mcg/mL, confirming ketoacidosis. LC-TOF identified presumptive cannabinoid positivity; no medications of toxicological significance were detected.

Discussion: The absence of blood ethanol alongside highly elevated VH and stable urine ethanol cannot be resolved through alcohol analyses alone. Progressive VH ethanol decline, rising isopropanol, and falling acetone across serial reanalyses indicate active postmortem biochemical transformation, while markedly elevated VH glucose provided substrate for microbial ethanol production during the postmortem interval. BHB at 920 mcg/mL unambiguously confirms ketoacidosis. Combined with reported cachexia, chronic alcohol use, and clinical decline in

the month preceding death, the findings support diabetic ketoacidosis and raise the possibility of concurrent alcoholic and starvation ketoacidosis, although the relative contribution of each process cannot be determined. Each pathway independently elevates ketone bodies and can generate endogenous acetone and, through microbial or chemical reduction, isopropanol and potentially ethanol — collectively accounting for the observed inter-matrix discordance. Discordant ethanol findings should prompt investigation beyond standard panels. BHB quantification should be incorporated into postmortem protocols when ketoacidosis is clinically suspected, and thorough collateral history encompassing treatment compliance, nutritional status, and substance use remains indispensable for accurate toxicological interpretation and determination of cause and manner of death.

Disclosure

Yes, I, or a member of my immediate family, has a financial interest to disclose.

Conflict of Interest

Salary

O-091

Warning from Madrid on the Dangers of Chemsex: A Toxicological Cohort Study of 35 Autopsy Cases (2017-2025)

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Abstract

Introduction: The term chemsex has appeared within the media and scientific literature within the past decade. It has been defined as long and intensive sex sessions with multiple partners, under the influence of a combination of psychoactive drugs, mainly GHB, methamphetamine (crystal), cocaine, alkyl-nitrites ("poppers"), mephedrone, sildenafil, etc. Subjects are reported to be mostly, but not exclusively, men who have sex with other men.

Chemsex participants have reported psychiatric health issues such as depression and psychosis, and a high prevalence of sexually transmitted infections due to high-risk behaviours.

Objectives: A review of the literature in PubMed, keywords "chemsex" and "postmortem" shows very scarce studies. It was the reason to consider its scientific interest.

This work evaluates all forensic aspects from 35 autopsy cases occurred in Madrid and surrounders (2017-2025), considered within chemsex, including histories, toxicological findings, and medico-legal interpretations. Samples were submitted for toxicological analysis to our Institution.

Methods: Toxicological analyses were carried out on blood, urine, vitreous, and paraphernalia, using conventional methods. GC-HS-FID for volatiles. CEDIA for common drugs prior to confirmatory detection in urine. GC-MSMS for cannabinoids in blood. GC-MS for THC-COOH in urine. LC-MSMS multi-target-screening for drugs and metabolites in blood, urine, and vitreous, LLOQ: 5 ng/mL (blood and vitreous) and LOD: 10 ng/mL (urine), in general. LC–HR-MS-MS-Orbitrap for complementary screening. Paraphernalia by GC-MS. Blood methemoglobinemia % using a UV-VIS-spectrophotometer.

Results: Each case (n = 35) involved report of using drugs with sexual activity. Age range: 22-61 years (median, 41). The males (n=34) died after pre-arranged meetings with other males or engaging in sex acts alone, including one in a sex-shop-cabine. The female died from asphyxiation by strangulation with a ligature after chemsex sadomasochistic practices with her husband.

Most detected substances and concentration ranges: all cathinones: 60.0%, n=21, individually: 2-MMC: 22.9%, n = 8-0, 01-2.72 mg/L; 3-MMC: 17.1 %, n=6, 0.02-2.0 mg/L; 3-CMC: 8.6%, n=3, 0.01-0.30 mg/L; 4-MEC: 5.7%, n=2, 0.01-0.41 mg/L; 4-MMC (mephedrone): 8.6%, n=3, 0.18-0.90 mg/L; GHB (>50mg/L): 54.3%, n = 19, 53.1-2216.0 mg/L; sildenafil/tadalafil: 45.7 %, n = 16, sildenafil 0.01-0.67 mg/L/tadalafil 0.02-1.23 mg/L; methamphetamine: 37.1%, n = 13, 0.01-0.54 mg/L; alcohol: 28.6 %, n = 10, 0.13-3.07 g/L; cocaine: 28.6%, n = 10, 0.03-1.15 mg/L, alkyl-nitrites "poppers": 28.6%, n=10, MetHb: 3%-68%.

Others substances were: common abused drugs, benzodiazepines, and antiretrovirals.

Discussion: The seven most detected drugs or type of drugs are: cathinones, GHB, sildenafil/tadalafil, methamphetamine, alcohol, cocaine, and poppers. Cathinones and sildenafil/tadalafil was the most common combination (31.4%, n = 11) followed by cathinones and GHB (28.6%, n = 10).

Cause of death was mostly polydrug intoxication but one maligne hyperthermia, two myocardial infarctions, two autoerotic hanging, and autoerotic asphyxiation (the woman). Manner of death was mainly accidental; the woman was suspected of having been sexually abused.

Polydrug use commonly associated with chemsex, creates highly toxic drug interactions and overdose with unconsciousness and death. Users of these psychostimulants and sex enhancing mixtures of drugs seldom realize the serious health hazard. This is the first study providing toxicology data in a series of chemsex fatalities occurred in Spain.

Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

O-092

From Evidence to Artifact? Matrix-Dependent Drug Detection and Formalin-Associated Redistribution in Fixed Postmortem Tissues

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Abstract

Introduction: Formalin-fixed (10% buffered, pH 7) and paraffin-embedded (FFPE) tissues may serve as alternative matrices in retrospective forensic toxicological investigations, particularly when reanalysis is required but preferred biological specimens such as postmortem blood are no longer available. However, the effects of fixation and embedding on drug recoverability and toxicological interpretation remain insufficiently characterized.

Objectives: This study evaluates matrix-dependent differences in drug detection and investigates potential formalin-associated redistribution in a forensic case analyzed six months after autopsy.

Methods: Postmortem blood analysis identified 2-MMC (16 ng/mL), a-PVP (111 ng/mL), clozapine (3647 ng/mL), N-desmethylclozapine (976 ng/mL) and metformin (123478 ng/mL). Six months later, brain, lung, kidney, heart (Sample A) and liver (Sample B) tissues preserved in formalin and as FFPE blocks were analyzed by LC–MS/MS, and their formalin fixatives (from samples A and B) were also examined.

Results: Clear matrix-dependent differences were observed. 2-MMC and metformin were not detected in fixed tissues. a-PVP was consistently detected in formalin-fixed tissues (1.2–2.1 ng/g) and in the formalin fixative (4.1 ng/mL – sample A and 2.9 ng/mL – sample B), but was absent in FFPE samples.

Discussion: The relatively homogeneous concentrations observed across organs, together with detection in the fixative, are consistent with possible post-collection diffusion or leaching into the formalin fixative. Clozapine showed markedly higher concentrations in formalin-fixed tissues (33–396 ng/g) compared with FFPE samples (0.3–1.6 ng/g), indicating significant analyte loss during paraffin embedding. N-desmethylclozapine showed similar but less pronounced matrix-dependent differences.

These preliminary studies demonstrate that drug detection in fixed tissues is strongly matrix-dependent and may be significantly influenced by fixation and embedding processes. Detection of a-PVP in the formalin fixative highlights the potential for formalin-associated redistribution, which may alter the apparent postmortem distribution pattern. Fixed tissue can be an alternative matrix for some compounds but its results should therefore be interpreted with caution and should not be considered directly equivalent to fresh postmortem specimens when drawing toxicological interpretations.

Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

O-093

Postmortem Blood Lead Profiles in Non-Firearm Related Deaths and Firearm Related Deaths

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Abstract

Introduction: Lead (Pb) is an element of antiquity; its history can be traced to the Neolithic Age. The pharmacokinetics and pharmacodynamics of Pb in living humans has been extensively studied; however, pharmacological studies involving Pb in deceased individuals is limited. Initially, the aim of our work was to generate data for possible addition to the sparse published collection of postmortem blood Pb reference points. We sought to characterize postmortem blood Pb concentrations in decedents having lived in an area with a well-documented history of lead contaminated drinking water. Among this cohort we encountered a case where the decedent's postmortem blood Pb concentration was magnitudes higher than what would be compatible with life; surprisingly, the decedent's cause of death was a shotgun wound.

Objectives: In response to the aforementioned firearm related death, we designed an ancillary study to evaluate blood Pb concentrations of decedents that were victims of fatal gunshot injuries. We evaluated postmortem blood from 124 non-firearm related deaths and 124 firearm related deaths from various counties in Michigan. Additionally, we examined the possible dispersal of Pb during gunfire in the setting of close contact by examining vitreous fluid from 24 decedents having gunshot injuries of the head. In this study, we took note of the sample collection site, projectile wound site(s), ammunition type, and the type of firearm used to cause the firearm-related injury.

Methods: The validation method performed for this work was compliant with the guidance set forth by ANSI/ASB Standard 036 and for some aspects of the validation, our practices exceeded said guidance. Postmortem blood (Femoral, Iliac, Subclavian, Heart, Cavity) and Vitreous Fluid was homogenized with a matrix modifier (Ammonium dihydrogen phosphate, Triton X-100, and Nitric Acid) to 1) reduce the volatility of Pb thus facilitating pyrolysis at temperatures where the loss of Pb would typically occur, 2) to increase the volatility of the sample matrix to drive off concomitants that may cause interference during atomization, and 3) to reduce background absorption. Samples were analyzed via Graphite Furnace Atomic Absorption Spectroscopy, a highly sensitive analytical technique that makes use of a graphite tube (GT) inserted in the sampling compartment of an atomic absorption spectrometer. The GT has a light aperture that allows light to pass through. Sample is quantitatively dispensed onto the GT and subsequently heated to a prescribed temperature. The heated sample is dissociated into atoms which diffuse from the GT causing changes in absorbance that represent a quantifiable analytical signal.

Results: Mean blood Pb concentration for non-firearm deaths was 0.72 mcg/dL, range 0.70 to 2.45 mcg/dL. Mean blood Pb concentration for firearm deaths was 20.2 mcg/dL, range 0.70 to 1096 mcg/dL. No detectable concentrations of Pb were observed in vitreous fluid samples.

Discussion: Blood Pb concentrations were typically lower for decedents injured with full metal jacketed ammunition as compared to decedents injured with un-jacketed lead projectiles. Notable differences in blood Pb concentrations across the site of gunshot injury were also observed.

Our findings demonstrate a correlation between postmortem blood Pb concentrations, specimen collection site, ammunition type, projectile wound site, and type of firearm used.

Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

O-094

Fatal EDTA Toxicity Following Chelation Therapy

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Abstract

Introduction: A 31-year-old woman developed seizures during chelation therapy administered as part of an aesthetic/wellness treatment involving intravenous infusion of disodium EDTA. She subsequently experienced cardiac arrest with pulseless electrical activity (PEA) and required extracorporeal membrane oxygenation (ECMO) and renal replacement therapy in the intensive care unit. Clinically, she had persistent hypocalcemia and evidence of multi-organ failure. Neuroimaging and electroencephalography demonstrated hypoxic–ischaemic encephalopathy. She died five days later. An antemortem serum sample, collected on the day of collapse for routine biochemistry testing, was later seized during police investigation for toxicological analysis.

Objectives: This study aimed to develop and validate a liquid chromatography–tandem mass spectrometry (LC–MS/MS) method to detect and quantify EDTA in serum, and to determine its concentration in this case.

Methods: Analysis was performed using a Shimadzu 8030 LC–MS/MS system with electrospray ionisation in positive mode. Sample preparation consisted of protein precipitation with cold acetonitrile using 0.1 mL of diluted serum, with EDTA-d16 as the internal standard. Chromatographic separation was achieved using isocratic elution with water/acetonitrile (95:5) containing 0.6% ammonia (pH ~8) on an Allure PFP Propyl column (50 × 2.1 mm, 5 µm) at a flow rate of 0.3 mL/min. Multiple reaction monitoring (MRM) transitions for EDTA were m/z 293 → 160, 132, and 56. Although EDTA-d16 has a nominal molecular mass of 308 Da, hydrogen–deuterium exchange of the carboxyl deuteriums in aqueous media results in a predominant molecular mass of 304 Da; therefore, the MRM transitions monitored were m/z 305 → 168 and 140.

Results: Significant signal enhancement was observed for both EDTA and the internal standard. Additionally, low levels of EDTA were detected in commercial blank blood, possibly as an anticoagulant. Thus, a standard addition approach was employed for quantification. Calibration using four different added concentrations from 2.5 to 10 µg/ml demonstrated good linearity (R^2 = 0.9988). The method has a limit of detection of 0.15 µg/ml. The EDTA concentration in the antemortem serum sample was determined to be 89 µg/ml.

Discussion: EDTA is not endogenously produced but is widely present in food products, pharmaceuticals, and cosmetics as a stabilising agent. Typical human plasma was reported to contain only trace levels of EDTA (reported at <0.015 µg/ml). Data on toxic or fatal ETDA level remain rather limited. In this case, the elevated EDTA concentration, together with severe hypocalcemia and the clinical course, supports EDTA toxicity as the cause of death.

Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

O-095

Prevalence of Naloxone with Opioids and Non-Opioids in Dallas County

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Abstract

Introduction: Naloxone, the active ingredient in Narcan® Nasal Spray, is a competitive opioid antagonist that reverses an opioid overdose. Naloxone availability has changed over the last ten years to help combat the opioid epidemic. Narcan® Nasal Spray was first approved in 2015 by the Food and Drug Administration (FDA) through a prescription. In 2023, the FDA approved for over-the-counter sale. The ease of access and administration of Narcan® allows trained personnel (e.g., emergency services, hospitals) and friends and family to administer the nasal spray in the event of an opioid overdose.

The Toxicology Laboratory at the Dallas County Southwestern Institute of Forensic Sciences (SWIFS) has been quantitating naloxone by liquid chromatography tandem mass spectrometry (LC-MS/MS) since 2017. The method also quantitates opioids, including fentanyl, 6-monoacetylmorphine (6-MAM), and morphine. However, there are consistently cases where naloxone was the only drug reported from this assay, sparking the curiosity of what other drugs naloxone has been reported with.

Objectives: To assess the prevalence of naloxone and co-occurrence of opioid and non-opioid drugs in toxicological casework.

Methods: Naloxone results from toxicological casework submitted to SWIFS since the start of naloxone quantitation (June 30, 2017) to December 31, 2025, were reviewed. If a case was positive for naloxone, in any matrix analyzed, the case was further reviewed for the presence or absence of an opioid. If no opioid was identified, the presence of other drugs in the case was reviewed. In postmortem casework, where the only toxicological finding was naloxone, cause and manner of death determinations were reviewed.

Results: From June 30, 2017, to December 31, 2025, the Toxicology Laboratory reported naloxone in 2,390 cases: 19 were DWIs, 18 were classified as “Other”, and the remaining 2,353 were postmortem cases. The number of naloxone positive cases, by year, ranged from 77 (2017) to 382 (2022). Reported naloxone concentrations in blood ranged from 1.0 – 929.0 ng/mL (average: 29.1 ng/mL; median: 14.7 ng/mL; calibration range: 1.0 – 100.0 ng/mL).

Naloxone was identified with fentanyl in 595 cases (25%); naloxone and fentanyl were the only drugs identified in 287 of the 595 cases. Naloxone was identified with another opioid (excluding fentanyl) in 312 cases. There were 1,281 cases (54%) where naloxone was reported from the opioid assay without the presence of an opioid.

The 1,281 cases were reviewed for prevalence of non-opioid drugs with naloxone and to identify the number of naloxone only cases (Table 1). No additional evaluation was performed on the cases where an opioid was identified. The medical examiners ruled the manner of death as “natural” in 145 of the 172 naloxone-only cases (84%) with cardiovascular disease, hypertension, and arrhythmias frequently cited as the cause of death.

Table 1. Frequency of drugs identified with naloxone.

Naloxone Drug Combinations	Count
Total Naloxone Reports	2,390
Opioids	
Fentanyl/Norfentanyl (alone)	287
Fentanyl/Norfentanyl (with other drugs)	308
6-MAM (with other drugs)	125
Other opiates/opioids	187
Non-Opioids*	
Ethanol	300
Methamphetamine	232
Amphetamine	215
THC and Metabolites	152
Cocaine and Metabolites	139
Amiodarone	68
Gabapentin	29
Atropine	28
Lidocaine	18
Metformin	14
Naloxone Only	172

*a case may be counted more than once

Discussion: Naloxone is a known resuscitation drug administered to reverse the effects of an opioid overdose. Since SWIFS began reporting naloxone, there has been a steady increase in the number of naloxone cases from 2017 to 2025, with a peak in 2022. The number of cases where naloxone was reported in the absence of an opioid has remained higher each year compared to the number of cases where naloxone was reported with an opioid.

Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

O-096

Toxicological Evaluation of Fentanyl-Involved Pediatric Homicides

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Abstract

Introduction: Fentanyl-involved deaths in the pediatric population are rare and present a significant challenge to forensic pathologists determining cause and manner of death. Exposure to fentanyl raises concerns of child endangerment not only in relation to the death investigation but to siblings in the same household. Toxicological investigations are critical but can be compromised in the pediatric population, due to a lack of specimen volume, limited collection or availability of different specimen types, the role of postmortem redistribution and the impact from resuscitative efforts.

Determining the route of administration is not without its challenges. The analysis of multiple specimen types including gastric contents provides greater insight into the distribution of fentanyl. With oral exposure being the primary source of fentanyl-involved pediatric homicides, testing gastric contents can aid in determining the route of administration.

Objectives: To evaluate the value of testing multiple specimen types, including gastric contents, in fentanyl-involved pediatric homicides to determine the route of administration.

Methods: In-house databases were retrospectively searched over a five-year period from 2021 – 2025 for pediatric deaths where fentanyl was listed in the cause of death, ruling out those where fentanyl was used during medical intervention.

Multiple specimen types, including blood, vitreous humor, urine, liver and gastric contents were tested for fentanyl, its metabolites and illicit analogs by validated methods utilizing liquid chromatography tandem mass spectrometry. Routine toxicological tests were also completed and reported. Gastric contents (total volume) were homogenized, after the removal of pills where applicable, and milligram content calculated based on the total gastric weight submitted.

Results: Twelve fentanyl-involved pediatric cases were identified where the manner of death was homicide. In all cases the cause of death was determined as acute intoxication. Ages ranged from 2 months to 5 years and one case involved a fetus of approximately 39 to 42 weeks, that survived 7 hours.

In addition to fentanyl and its metabolite norfentanyl, the following illicit drugs/metabolites were detected in blood: acetylfentanyl, fluorofentanyl, acrylfentanyl, 4-ANPP, β-hydroxyfentanyl, n-methyl norfentanyl, 6-monoacetylmorphine, morphine, xylazine, medetomidine, cocaine, benzoylecgonine, bromazolam and cannabinoids.

Femoral blood (N=6) concentrations ranged from 0.15 to 54 ng/mL for fentanyl and from 11 to 1600 ng/mL in heart blood (N=6). Gastric content fentanyl concentrations ranged from 1600 to 27000 ng/mL and from 0.06 to 1.6 mg corrected for total weight.

Discussion: Although there are toxicology publications that discuss pediatric fentanyl exposure, limited studies include quantitation in gastric contents. Taking into account the circumstances of the cases in this study, positive gastric contents supported ingestion as the route of administration.

In one case, where both the femoral blood (1600 ng/mL) and gastric contents (27000 ng/mL) were highest, EMS found the decedent in full rigor mortis but cardiopulmonary resuscitation (CPR) efforts were attempted. Many factors influence postmortem redistribution (PMR) and this may also include CPR. This must be considered when interpreting the very high concentrations in this case.

Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

O-097

Data Mining with OpenMS for the Retrospective Identification of Out-of-Scope Analytes

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Abstract

Introduction: The continual emergence of multiple classes of novel psychoactive substances (NPS) has driven forensic chemists and toxicologists towards untargeted high-resolution mass spectrometry acquisition methods. Analysis of the data is usually targeted and limited to a specific scope or database. Although this approach ignores the abundance of data in the original file, it leaves an archive of data that can later be mined for analytes out-of-scope at the time of the original testing. Retrospective data analysis is a powerful drug surveillance tool; however, its full potential is often under-utilized due to high computational demands and labor-intensive workflows that create a significant barrier to routine implementation.

Objectives: A data mining program was developed using the OpenMS framework to facilitate offline retrospective analyses of liquid chromatography high-resolution mass spectrometry (LC-HRMS) data. Emphasis was placed on reducing computational bottlenecks and streamlining mass review of complex historical datasets to permit rapid iterative reanalysis of the original data for the identification of emerging drugs.

Methods: The original *.d* data files, acquired on 6500 Agilent time-of-flight (TOF) instruments using Mass Hunter, were converted to the open-source *.mzML* file format using the Proteowizard MS Convert tool. A feature detection workflow was developed in The OpenMS PiPeline Assistant (TOPPAS) with parameters for modeling peak integration and isotopic patterns optimized against a library of over 200 common drugs of abuse, pharmaceuticals, cutting agents, and established NPS. The workflow was encoded in Python using the pyOpenMS library and deployed locally via a Streamlit browser application. Criteria for reporting were developed with the objective of reducing false positive identifications. A library of thirty-four out-of-scope emerging NPS was curated comprising six stimulants, three mitragynine alkaloids, eight benzodiazepine-related substances, eleven orphines, two nitazenes, one opioid antagonist, and three miscellaneous analytes. Blind testing was performed using blood and urine samples independently spiked with target compounds to assess detection parameters and positivity criteria. Postmortem blood and urine specimens previously analyzed over a six-month period (July-December 2025) were selected for mining and a queue system was established to automate data processing through the workflow.

Results: Retrospective LC-HRMS data mining is a valuable complement to forensic toxicology screening, expanding detection capabilities beyond those available at the time of original analysis. A six-month case archive (>56,000 total data files) was re-processed in 43.95 computational hours, yielding about 4400 out-of-scope identifications. A key advantage of the workflow is its data reduction capability. Data files yielding no library identifications are automatically excluded from reporting, substantially reducing the volume of data requiring final review and allowing analysts to focus attention exclusively on files with presumptive detections.

Discussion: Future work will focus on expanding the target library, sample scope, instrumentation platforms, and mass-spectral data types. While HRMS instrumentation meets the hardware demands of the field, the absence of efficient software and data processing solutions continues to limit its full potential. The OpenMS framework provides a flexible, open-source foundation that can be adapted to a variety of instrument platforms and method conditions.

Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

O-098

Development and Implementation of an In-House Built and Customized Laboratory Information Management System

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Abstract

Introduction: A laboratory information management system (LIMS) is a key component of any laboratory. In our field, there are numerous vendors that each offer slightly different versions of LIMS. Across other disciplines, users have found that commercial LIMS software does not address all the needs of the laboratory. Prior to 2025, the San Francisco Office of the Chief Medical Examiner (OCME) used a combination of commercial LIMS, a Microsoft Access database, Excel workbooks, and paper records to facilitate case management.

Objectives: The objectives of this project were to develop, implement and maintain a customized LIMS for the OCME, that also ensured sustainability over time.

Methods: The OCME LIMS was developed using a Microsoft SQL server with a python backend, HTML and JavaScript frontend and a Flask framework. The application is served on the City and County of San Francisco intranet using Nginx. Development was performed by an in-house team utilizing a maximum six team members during some periods, consisting of forensic toxicology laboratory experience, computer science background and bioinformatics backgrounds.

Results: Following implementation of the customized LIMS on January 1, 2025, the OCME has generated over 3800 reports, has facilitated over 750 batches, and has captured over 75,000 positive results. Customized LIMS automations saved the OCME over 1000 hours of staff time in just one year.

Discussion: The implementation of a custom LIMS has allowed the OCME to perform routine operations at a much more efficient rate. Investing in the time required for traditional scientists to learn how to code has resulted in a LIMS maintained by an in-house team of traditional and computer scientists. The continued development and improvement of the LIMS is efficient with bug fixes pushed out the same day they are identified. Further, the use of open-source and publicly available resources (Python, flask, JavaScript, etc.) allows the project to maintain sustainability beyond the contribution of any individual in-house developer additionally, without the fear of outside vendors increasing prices or going out of business. The ability for in-house developers to constantly collaborate on this project with everyday users allows the LIMS to continue to grow.

Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

O-099

Multimodal Bayesian Retrieval of Obscured Drug-Related Content for Forensic Informatics and Clinical Toxicology

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Abstract

Introduction: The rapid spread of drug-related content on social media presents growing challenges for both forensic informatics and clinical toxicology. Substance-related posts may contain signals relevant to emerging drug trends, patterns of misuse, polysubstance exposure, and behavioral risk, yet these signals are often obscured through coded language, slang, emojis, indirect references, and other forms of adversarial vocabulary mismatch. In multimodal platforms such as TikTok, YouTube, and Reddit, meaning is distributed across speech, captions, comments, and on-screen text, making conventional keyword-based retrieval insufficient for identifying clinically and forensically relevant content.

Objectives: To address this problem, we present a Bayesian evidential reasoning framework for multimodal information retrieval designed to improve the detection of semantically obscured drug-related content. This multimodal content-to-text framework supports evidential grounding by using modality-specific text signals as proxies for a unified semantic representation of post meaning.

Methods: Our approach models the relationship between observable evidence extracted from multimodal sources—including automated speech recognition (ASR), optical character recognition (OCR), captions, and user-generated text—and latent behavioral variables that reflect concealed substance-related intent. The framework further integrates structured Object-Attribute-Value (OAV) extraction and substance lexicon identification to support retrieval, ranking, and interpretation of relevant content.

Results: We evaluated the approach using an iterative seed-to-stream expansion strategy that began with a 300-video TikTok seed set and produced a dataset of 1,200 expert-labeled posts across four graded relevance categories. This annotated dataset was then used to generate semantically informed queries for retrieval across an automatically harvested cross-platform corpus of 20,000 posts from TikTok, YouTube, and Reddit.

Discussion: Performance was assessed using nDCG@k, precision, and F1-score. Results demonstrate that the proposed multimodal learning-to-rank framework outperforms text-only and LLM-as-classifier baselines in retrieving euphemistic, behaviorally relevant, and substance-related content. Ablation studies further show the value of multimodal language model–assisted query refinement and feature fusion centered on multimodal text capture. These findings highlight the potential of evidentially grounded retrieval methods to strengthen toxicology surveillance, support early signal detection, and enhance forensic informatics workflows for monitoring evolving substance-use behaviors in digital environments for use by policy makers, law enforcement, and forensic laboratories.

Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

O-100

Implementation of Artificial Intelligence Tools in a Forensic Toxicology Laboratory: Enhancing Efficiency and Quality Management

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Abstract

Introduction: Over the past few years, the Laboratory of Toxicological Analysis at the Jan Sehn Institute of Forensic Research in Krakow has systematically integrated Artificial Intelligence (AI) tools to optimize forensic casework and laboratory management. These implementations range from administrative support to complex analytical processes and rigorous quality control, aiming to modernize traditional forensic workflows.

Objectives: The primary objective of this AI implementation is the reduction of human error within the Quality Management System (QMS) and the enhancement of the reliability of identifying complex toxicological targets. The goal was to create a flexible, integrated system that supports both administrative tasks and advanced analytical identification while maintaining the highest standards of data security.

Methods: The laboratory utilized Large Language Models (LLMs), such as OpenAI's GPT-4 and Google's Gemini Pro, as programming assistants for script languages (PHP, Python), and HTML. This facilitated the development of a "homemade LIMS" (Laboratory Information Management System) by transforming existing scripts into an integrated platform. For analytical purposes, proprietary Machine Learning Algorithms (MLAs) were implemented for substance identification using LC-QTOF, based on multi-parametric analysis of accurate mass and retention time stability. To ensure data integrity, sensitive information is processed using LLMs in an offline environment (local deployment).

Results: The integration resulted in the successful creation of a highly flexible "homemade LIMS" and the automation of administrative tasks, including scientific summarization and infographic generation. The application of AI tools led to a significant reduction in clerical errors (e.g., incorrect units, inconsistent evidence descriptions) and improved consistency between raw instrumental data and final expert opinions. The AI-supported LC-QTOF approach significantly increased the precision of identifying complex toxicological substances.

Discussion: The custom-built system offers a high degree of flexibility and expandability compared to commercial solutions. However, local deployment of LLMs presents challenges, specifically the need for substantial hardware investment (high-end graphic processing units - GPUs). It was determined that for forensic reliability, models should have at least 14 billion parameters (e.g., Qwen3:14B). Crucially, these tools do not replace human experts but serve as an auxiliary layer of oversight, ensuring full compliance with ISO 17025 requirements through "human-in-the-loop" verification protocols.

Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

O-101

Chromatographic Peak Detection and Integration by Supervised Machine Learning using Convolutional Neural Networks

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Abstract

Introduction: Positive or negative samples in forensic toxicology casework are determined by the presence, or absence, of peaks in chromatograms generated by chromatography mass spectrometry instrumentation. Peak detection and integration are undertaken by instrument vendors' software algorithms. On occasion, these algorithms perform poorly such as incorrectly selecting a nearby peak or drawing an improper baseline. Consequently, all data needs to be routinely processed, checked and reviewed by a human. This is time and resource heavy with casework batches containing multiple samples analyzing multiple drugs. Peak integration can be a highly subjective process where patterns (signal) are determined against a complex background of noise. This can be readily misinterpreted by humans or be too complex to be accurately and reproducibly solved by simple algorithms. Machine learning has the potential to detect and integrate peaks in a way that is less subjective than manual human intervention and could be a better alternative to the vendor's software. This would save both time and resources at the data processing stage as human would still undertake the final checking.

Objectives: The aim was to develop a novel peak integration and data processing platform based on machine learning to overcome the instrument vendors software limitations and increase efficiency of the data processing stage of casework workflows.

Methods: Retention times and integration start/end points from 2.8 million peaks (1,800 batches) were extracted from Shimadzu's LabSolutions .lcd acquisition data and Insight .iproc processed data files and consolidated into Python objects. An in-house synthetic peak generator was also used to augment the dataset. Neural networks producing peak integrations were built and trained using Google's TensorFlow. A model that predicts peak location and integration start/end coordinates concurrently, the unified multi-output regression (UMOR) model, was divided into three separate single-output regression (SOR) models that predict each of these coordinates separately. The architecture was further refined by shifting to spatially aware probabilistic classification (SAP) models that produce a probability prediction for peak location and integration along the entirety of the acquisition window and finally feature-enhanced SAP (FESAP) models that use input data augmented with classical mathematical algorithms. Whilst training was a fully automated supervised process using million-sized and hundred-thousand-sized datasets for testing and validation respectively, the models' final performance was evaluated by humans.

Results: The splitting of UMOR models into SORs yielded significant improvement in peak integration accuracy. SAP models also provided insight into the peaks' morphological features that models prioritise. FESAPs, especially when using derivatives of the original data, showed even better ability to integrate poorly shaped peaks. In some instances, the models provided better integration than originally performed by a human.

Discussion: Using such a large dataset helps not only to prevent overfitting but also allows the model to converge on a human-like consensus as to where the integration starts and ends. This is helpful since there is no absolute mathematical way to determine what the correct integration of a peak is. The resulting algorithm shows less subjectivity than a human and greater accuracy than vendor's software. It was built into in-house data processing software that processes batches with minimal human intervention.

Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

O-102

PhaT-GPT (Pharmacological Toxicity Assessment by Global Pill Testing): Application of Activity-Based Opioid Screening in Drug Checking

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Abstract

Introduction: The rapid emergence of new synthetic opioids (NSOs) on the recreational drug market poses a major public health threat. Apart from intentional use, NSOs have also been detected in a variety of recreational drugs and counterfeit medications, which greatly increases the risk of intoxication and overdose. Drug-checking services play an important role, as these organizations can rapidly send out warnings about circulating pills or powders that are particularly dangerous. However, conventional toxicological screening may fail to detect newly emerging, unknown compounds. Furthermore, risk communication becomes increasingly complicated when dealing with mixed drug preparations.

Objectives: We set up a new drug testing initiative coined "PhaT-GPT" (Pharmacological Toxicity assessment by Global Pill Testing), which applies activity-based screening for the detection of opioid activity in authentic drug samples. To circumvent safety and regulatory complications during transport, the drug material is supplied as 'dried drug spots' (DDS). The primary objective was to extract the drug(s) from the DDS and apply a μ-opioid receptor (MOR) activation bioassay to identify (high-risk) opioid-positive samples. Secondly, we attempted a risk assessment of each opioid-positive sample by assigning so-called "fentanyl activity equivalents".

Methods: A total of 165 authentic drug samples, including 18 duplicates, were supplied by the Welsh drug-checking service WEDINOS in the form of methanolic solutions spotted on filter paper (DDS). Specifically, to generate DDS, 5 μL of a methanolic extract that was generated according to the routine pill/powder testing protocol at WEDINOS (i.e., by dissolving roughly 10 mg powder/pill homogenate in ~1 mL of methanol) was spotted on filter paper. After transport, samples (full spots) were extracted in 24-well plates for 1 hour using 500 μL of 80% methanol/20% OptiMEM® (v/v). The resulting extracts were 5-fold diluted with OptiMEM® prior to applying 20 μL to an in-house developed, *in vitro* MOR bioassay to screen for the presence of opioids.

Results: Using a blind-coded approach, the results of the activity-based screening (opioid positive/negative) were compared to qualitative (LC-QTOF) findings by WEDINOS. Twenty-nine samples were correctly scored positive; 102 opioid-negative samples were correctly scored as such. The sensitivity and specificity were 81% and 92%, respectively. Of the 7 false negative samples, 4 contained tramadol, a weakly active opioid agonist; hence, these samples were not considered 'high risk'. All duplicate samples showed consistent results. As also a fentanyl calibration curve could be set up from in-house generated DDS, this allowed to assign fentanyl activity equivalents to opioid-positive test samples. As such, the individual samples could be stratified by risk based on the overall opioid activity present, with samples containing nitazenes giving the highest fentanyl equivalents.

Discussion: The results convincingly show the potential of PhaT-GPT as a universal screening method applicable for drug testing. We showed that DDS provide a convenient means of transport of potentially high-risk drug material. By assigning a general 'opioid risk score' based on fentanyl activity equivalents, drug material can be rapidly risk assessed, regardless of which (opioid) drug or combination of drugs is present in the sample. While further validation is needed, PhaT-GPT may significantly contribute to harm reduction by identifying dangerous drug material (e.g. containing nitazenes) in circulation.

Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

O-103

Navigating the New Frontier: Method Development Challenges in Fast Oral Fluid Analysis by DART-MS/MS

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Abstract

Introduction: The detection of illicit substances in oral fluid (OF) has become a key element in roadside drug testing. In France, the December 2016 decree defines the legal cut-offs for ten target analytes including cannabinoids (tetrahydrocannabinol: THC), cocaine and its metabolite benzoylecgonine, opiates (6-MAM and morphine) and amphetamines (Amphetamine, MA, MDA, MDMA, MDEA). Following a positive roadside screening test, OF is collected using a FloqSwab® device and sent to an accredited laboratory for confirmatory analysis, usually performed by liquid chromatography coupled to tandem mass spectrometry or high-resolution mass spectrometry (LC-MS/MS or LC/HRMS). While robust, LC-MS/MS is constrained by long run times, solvent use, carry-over, and maintenance, Direct Analysis in Real Time tandem mass spectrometry (DART-MS/MS) offers a chromatography-free approach and faster alternative.

Objectives: This abstract presents the challenges encountered during the development of an analytical method based on a novel technology, namely DART-MS/MS for the rapid screening and quantification of the drugs regulated by the French legislation in oral fluid collected using FloqSwab® devices.

Methods: Analytes were extracted from FloqSwab® devices using Quantisal® buffer followed by liquid-liquid extraction. The extract was then split into two fractions for cocaine-related-opiates-amphetamines, and the other for cannabinoids. After evaporation, the cannabinoid fraction was derivatized, whereas the other was reconstituted in methanol. Aliquots of 5 µL were deposited onto a QuickStrip™ grid and analyzed by DART-MS/MS. Optimal gas heater temperatures were 130°C for amphetamines, 250°C for cocaine-related analytes and opiates, and 150°C for cannabinoids. The instrumental analysis time was below 60 seconds per sample.

Results: The legal cut-off concentrations of 1 ng/mL for THC and 10 ng/mL for opiates, cocaine-related analytes, and amphetamines were successfully achieved using neat oral fluid as the analytical matrix, without the FloqSwab® device. However, incorporation of the FloqSwab® during the analysis of authentic specimens resulted in substantial ion suppression, particularly affecting cannabinoids.

Discussion: Method development using DART-MS/MS highlighted several analytical challenges. The different desorption temperatures required multiple sample deposits, as desorption efficiency was strongly dependent on compound volatility and thermal behaviour. For example, amphetamines desorbed at approximately 130°C whereas opioids required temperatures close to 250°C.

As identification relies exclusively on the comparison of characteristic ion ratios, reproducibility is necessary. Since satisfactory performance was achieved for all analytes except cannabinoids, optimization efforts focused on this class.

Moreover, the absence of chromatographic separation limits discrimination of isobaric compounds unless derivatization is applied. This was particularly relevant for THC and cannabidiol (CBD), to allow unambiguous identification of THC in accordance with French legal requirements.

Initial silylation with MSTFA resulted in weak ion signals and marked ion suppression, particularly in authentic samples. Although an alternative silylation reagent improved ion ratio reproducibility, sensitivity remained insufficient. Further optimization strategies, including negative ionization, methylation, extraction optimization (Solid-Phase Extraction) may improve cannabinoid detection.

Overall, this work demonstrates that the implementation of a novel technology such as DART-MS/MS involves numerous method development challenges particularly for complex matrices and low cut-off analytes such as cannabinoids. Nevertheless, DART-MS/MS showed considerable potential for drugs in oral fluid.

Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

O-104

Automated R-Based Workflow for GC-MS Impurity Profiling: Application to Methamphetamine Datasets.

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Abstract

Introduction: Impurity profiling plays a critical role in understanding the synthetic routes, origins, and distribution patterns of illicit drugs. Gas chromatography-mass spectrometry (GC-MS) is widely used due to its sensitivity for trace level compounds. The effectiveness of GC-MS based impurity profiling however, depends on robust data processing and interpretation.

Chemometric methods enable classification, pattern recognition, and similarity comparison across drug samples. However, current approaches often rely on manual data processing and interpretation, which is time-consuming, labour intensive and limits scalability.

This research addresses these limitations by developing an automated, end-to-end computational pipeline, in R for impurity profile mapping.

Objectives: To develop and validate an automated R workflow for systematic impurity detection, ranking, and spectral matching in GC-MS datasets, designed to improve reproducibility and efficiency in forensic workflows.

Methods: Raw GC-MS data from 128 fully characterised methamphetamine samples were processed using custom R scripts incorporating baseline correction, smoothing, and automated peak detection (signal-to-noise ratio ≥ 3). Detected peaks by R were evaluated against manually validated reference dataset to assess concordance and completeness. Identified peaks were then ranked using an intensity-based approach to detect prominent impurities across samples.

Mass spectra for retained peaks were compared with a dynamically generated impurity library, iteratively expanded from the processed dataset using spectral contrast angle similarity measurements to support automated impurity mapping across samples.

Results: The automated approach detected 23,885 peaks across 128 chromatograms and successfully recovered all manually validated reference peaks (n = 1,531), demonstrating sensitivity to both known impurities and low-intensity peaks not represented in the reference dataset. Each signal was assigned a single representative retention time, enabling consistent peak characterisation across samples and highlighting the robustness of the approach in capturing impurities.

After ranking and filtering, 2,559 distinct impurity peaks were retained (~10% of total) maintaining full reference coverage, indicating that comprehensive analysis can be achieved using a substantially smaller subset of data, providing substantial time savings in practice. The number of top-ranked peaks required to achieve full coverage of reference peaks varied between synthetic routes, reflecting differences in impurity profiles and signal intensity distributions.

Spectral-angle matching enabled consistent peak assignment across samples, with similarity scores ranging from 0 (no similarity) to 1 (perfect match) and visualised using heatmap representations to facilitate comparison of impurity patterns.

Discussion: This study demonstrates that R-automated processing of large and complex GC-MS datasets can reliably detect, prioritise, and identify impurity peaks while substantially reducing manual workload and improving analytical consistency. The approach enables systematic and reproducible analysis of high-throughput data, addressing key limitations of manual peak interpretation.

Although validated using methamphetamine samples, the workflow is fully generalisable and provides a scalable framework for impurity profiling across diverse datasets, supporting efficient large-scale forensic data analysis. The outcomes of this approach have the potential to enhance forensic intelligence in drug profiling by supporting the identification of relationships between batches of seized drugs, thereby aiding investigative and operational decision-making.

Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

O-105

Assessing DRUID's Ability to Distinguish Between Alcohol and Cannabis-Induced Impairment Using Machine Learning

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Abstract

Introduction: DRUID® is a performance-based assessment tool, operating like a brief video game, that reliably detects cognitive-motor impairment resulting from fatigue, concussion, alcohol use, and cannabis, as documented in previous publications. Until now, DRUID has been used to identify impairment but not its *source*. However, there is increasing interest in determining not just impairment, but also its cause. Scientific literature indicates that different substances can affect cognitive and motor functions in distinct ways, influencing domains such as balance, reaction time, decision-making, time estimation, and motor control.

Objectives: This study evaluated whether DRUID could differentiate alcohol- and cannabis-induced impairment using machine learning applied to cognitive and psychomotor performance data.

Methods: DRUID is available either as a 1-minute test (DRUID *Rapid*) or a 3-minute test (DRUID *Benchmark*). For this study, we modified the DRUID *Benchmark* to create DRUID-*ai*, a research version with increased sampling frequency for selected performance indicators from 10 to 100 measurements per second without changing the app's fundamental algorithm. The increased data sampling frequency of DRUID-ai allowed us to collect more data points for each administered test.

The IRB-approved study enrolled 19 participants aged 21-50 years. Eighteen (9 Males, 9 Females) participated in two closely supervised sessions, one each for alcohol and cannabis; one additional participant served as a procedural control and received no substance. Participants established pre-dosing impairment scores using DRUID-ai and took a breath test for alcohol and an oral fluid THC point-of-care screen test before dosing. They consumed 80-proof vodka in the first session until reaching a BrAC of 0.10% or higher. On a separate day, they consumed cannabis ad libitum until reaching a self-reported impaired state. Post-dosing, a series of DRUID-ai tests was administered in duplicate at set intervals, yielding a total of 568 DRUID results across the two sessions.

Restricting the data sample to 210 post-dosing assessments within the first hour (when participants were at or near peak impairment) and then averaging the two tests at each time point yielded 105 data points from 17 participants for analysis (one participant's data were excluded because they had installed an incorrect app version). Using feature importance scores from initial Random Forest Classifier (RFC) fits, we identified 945 unique combinations of DRUID component scores. For each, we performed a grid-search cross-validation procedure to identify the optimal hyperparameters, then fit RFC 1,000 times and evaluated each model using a train/test split. Repeating this procedure yielded a total of 1,890 model configurations.

The RFC analyzed patterns in DRUID performance data (reaction time, balance, decision accuracy, etc.) and learned to classify whether the pattern of impairment was more consistent with alcohol or cannabis.

Results: Many of the machine-learning models tested were able to distinguish alcohol-related impairment from cannabis-related impairment with high accuracy. Of the 945 combinations evaluated, 315 produced median F1 scores exceeding 0.85 for both classes, indicating a strong balance of precision and recall.

Discussion: The results indicate that alcohol- and cannabis-induced impairment produce distinct data signatures that DRUID-ai can reliably differentiate. These findings suggest that DRUID-ai can characterize substance-specific impairment patterns and warrant further validation in larger, more diverse populations and impairment sources.

Disclosure

Yes, I, or a member of my immediate family, has a financial interest to disclose.

Conflict of Interest

I serve on the Board of Advisors for Impairment Science Inc., the company that developed DRUID.

O-106

Endogenous Sleepiness Markers Could Aid Forensic Routine DUI Case Interpretation

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Abstract

Introduction: Sleepiness detection remains one of the most challenging topics in forensic expert witness reporting in DUI cases, especially when no sleep-mediating drug is present. As sleep-wake regulation involves complex and concerted biological mechanisms, there is no single specific biomarker. Recently, our group has made promising progress via identification of twelve salivary metabolic sleepiness markers that were discovered in a clinical study, in combination with a predictive model that could aid in interpretation and classification.

Objectives: Since blood collection is mandatory in many countries, this work investigated whether metabolic markers of sleepiness are also present in blood and to what extent they can serve as supporting evidence in forensic DUI cases.

Methods: In total, 149 whole-blood specimens were analyzed via LC-MS in an untargeted metabolomics approach. 55 specimens from a clinical sleep study were used for important molecular feature detection of sleep-deprived and control subjects. 51 specimens were collected in routine forensic casework with a positive DUI decision for sleepiness based on either the individual's own statement (23), or single drug effect of benzodiazepine (12), zolpidem (10), or cocaine in late phase of its effects (6). 43 specimens served as negative controls matched for sex, age, and time of day for each positive case. An 8-fold cross-validated logistic regression model was trained on the study samples for classification of sleep-deprived vs. rested subjects and selection of the most predictive metabolites. This model was then used to retrospectively classify the routine DUI cases.

Results: Logistic regression identified three endogenous molecular features to be sufficient for sleepiness detection based on sleep history in the controlled study setting (AUROC = 0.86, accuracy = 0.87, precision = 0.84, F1 score = 0.80). A predictive model trained on these would have retrospectively supported the expert witness decision in 75% of sleepiness cases where no sleep drug was detected and blood sampling time matched training data. It further supported 67% of decisions in cases where any of the mentioned sleep-mediating drugs was detected. Outside the training data sampling time range, the model's support was limited. Interestingly, age, sex, or drug concentration showed no influencing trend on model sensitivity.

Discussion: Only few endogenous metabolic markers in blood were necessary to build a robust model that can reliably distinguish between sleep-deprived and rested subjects. Applied to real-world cases, collection time of day appeared to have the greatest impact on sensitivity. Therefore, a future forensic model for sleepiness detection would benefit from a large dataset of cases that were collected throughout the day. It should be noted that sleepiness caused by drug intake is likely to result in different molecular changes than those occurring under sustained wakefulness. Historically, the total number of positive sleepiness decisions by forensic experts is overall low compared to its prevalence due to precaution and restraint. In European jurisdictions,

toxicological proof is often weighted stronger than behavioral or circumstantial evidence, which may be different in other parts like the US. Nevertheless, sleepiness biomarkers could also aid here by adding another layer of certainty.

Disclosure

Yes, I, or a member of my immediate family, has a financial interest to disclose.

Conflict of Interest

MS and TK filed a patent for metabolic sleepiness markers in oral fluid.

O-107

Assistive vs Autonomous Applications of Artificial Intelligence and Machine Learning (AI/ML) in Forensic Toxicology: A U.S. Admissibility-Focused Framework Preserving Expert Judgment

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Abstract

Introduction: Integration of artificial intelligence and machine learning (AI/ML) into forensic toxicology (FT) presents unique challenges within the United States adversarial legal system, where scientific conclusions are routinely subjected to scrutiny through discovery, expert testimony, and constitutional confrontation rights. Under Confrontation Clause jurisprudence, including *Melendez-Diaz*, *Bullcoming*, and *Smith v. Arizona*, qualified forensic toxicologists must be able to explain and take ownership of testing processes, calculations, results, and interpretations presented in court. As AI/ML becomes integrated into laboratory operations and workflows, human experts must remain responsible for validation, quality assurance, interpretation, and final scientific conclusions. While international guidance has emphasized risk-based implementation and validation of AI/ML technologies, practical questions remain regarding -admissibility, accountability, transparency, and expert testimony in U.S. forensic practice.

Objectives: This work proposes a U.S.-focused framework for evaluating AI/ML implementation in forensic toxicology that prioritizes legally defensible applications while preserving the forensic toxicologist as the accountable scientific authority and testifying expert.

Methods: A structured narrative review was conducted integrating three domains: U.S. legal precedent, forensic science guidance documents and standards, and peer-reviewed literature describing current and emerging AI/ML applications. Representative applications were classified according to their proximity to the final forensic conclusion and evaluated for validation feasibility, explainability, human oversight, reproducibility, transparency, and anticipated admissibility under U.S. evidentiary standards. A risk-based framework was developed to classify AI/ML implementations according to their scientific and legal implications.

Results: Application of the framework identified three functional categories of AI/ML use in forensic toxicology: administrative and quality-assurance applications, assistive analytical and decision-support applications, and autonomous adaptive decision making systems. Administrative and quality-assurance tools, including chain-of-custody verification, data integrity checks, audit functions, and workflow automation, present the lowest admissibility risk because they do not generate forensic conclusions. Assistive analytical applications, including peak integration, spectral matching, quality-control assessment, retention-time prediction, HRMS data triage, and report drafting are generally consistent with current scientific practice when outputs are independently reviewed, accepted, and explained by qualified scientists. Defined decision-support systems occupy an intermediate position and may be acceptable when their operation, validation, limitations, and contribution to the final opinion can be articulated by the testifying expert. In contrast, autonomous adaptive systems that generate reportable findings or interpretive conclusions without meaningful human review present substantial challenges regarding validation, reproducibility, and transparency.

Unlike some other forensic disciplines, forensic analytical and interpretive toxicology routinely relies on complex instrumental data processing, library matching, chromatographic peak integration, HRMS screening workflows, and increasingly large retrospective datasets including interpretation based on previously reported data, makes the discipline particularly attractive for AI/ML implementation.

Discussion: The proposed framework mitigates risk of evidence exclusion with a practical basis for prioritizing AI/ML implementation projects, validation frameworks, data interpretation and testimony preparation according to anticipated scientific and legal challenges. Current U.S. legal frameworks place exceptional emphasis on the ability to challenge forensic evidence and expert testimony. Accordingly, AI/ML applications should assist rather than replace expert decision-making. A phased implementation strategy emphasizing validated administrative, quality-assurance, and assistive analytical applications offers the most practical pathway for responsible adoption while preserving scientific accountability, evidentiary reliability, and jurisprudential confidence in forensic toxicology.

Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

O-108

Python-Accelerated Processing of TraceFinder™ Method Validation Data per ANSI/ASB Standard 036

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Abstract

Introduction: While it is often assumed that data generation is the primary challenge of method validation, a more significant bottleneck can lie in data processing. Python is a high-level, general-purpose programming language available for laboratories to use. With novel psychoactive substances (NPS) emerging at a rapid rate, there is a clear advantage for laboratories to validate methods that can be deployed quickly. A collection of a few scripts can accomplish this task in a fraction of the time required by traditional methods.

Objectives: Efficiently extract and process multi-analyte panel validation data from raw data files using Python.

Methods: A suite of custom-coded scripts was developed for ThermoFisher Scientific™ TraceFinder™ software data outputs using Python v3.13. These scripts leveraged built-in Python libraries and those developed for data science (e.g., Matplotlib, StatsModels, Pandas, SciPy, and NumPy). Together, they automated data processing for the following key validation parameters: calibration model with weighted residuals evaluated per the Cramér–von Mises criterion, bias and precision data for the lower limit of quantitation and quality controls, dilution integrity, and ion suppression/enhancement.

Results: Validation data for four separate multi-analyte panels (opioids, cannabinoids, antipsychotics/antihistamines/antidepressants, and anticonvulsants and acidic/neutral drugs) were processed using the described Python scripts. The opioid panel had 43 total compounds, 14 of which were quantitative analytes. For this panel, the Python-based workflow reduced total analysis time of the validation parameters listed above from a likely several days to under 10 minutes. Validation data created by Python was compared to original validation data generated using the published Excel-based validation data processing tool EZSTATS[1]. For all four panels, the Python validation data matched the EZSTATS output.

Discussion: Advancements towards harnessing coding languages to facilitate aspects of data processing should be encouraged. Here, we have demonstrated how Python can be used effectively to meet critical ANSI/ASB Standard 036 requirements. The presented custom-coded Python scripts have demonstrated increased efficiency of validation data processing. The total processing time was reduced from possibly weeks, days, or hours to just a few minutes. By coding for data processing, we removed the technical inconsistencies and transcription errors associated with manual Excel-based workflows. While implemented in Python using TraceFinder™ data as a proof of concept, this toolkit can be modified to other programming languages and vendor data outputs to achieve similar processing efficiencies.

[1] Sofalvi, S., Schueler, H. E., & Abonamah, J. V. (2022). Expansion of the EZSTATSG1 Microsoft excel tool for ANSI/ASB standard 036 method validation by the addition of weighted quadratic external calibration models utilizing five-, six-or seven-point curves. *Journal of Analytical Toxicology*, 46(8), 925-931.

Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

O-109

Emerging Threats in Synthetic Opioids: A Case of Fatal Intoxication Involving Spirochlorphine (R-6890)

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Abstract

Introduction: Novel synthetic opioids (NSOs) are often considered the most dangerous subclass of new psychoactive substances (NPS) due to the high risk of fatal overdose. Since 2019, a new group of structurally diverse, non-fentanyl related NSOs collectively referred to as *orphines*, has emerged on the European drug market. Orphine-type opioids are highly potent synthetic compounds whose abuse can result in severe adverse effects like respiratory depression. Spirochlorphine, also known as R-6890, first appeared on the drug market in late 2024 and is structurally distinct from other NSOs within this group due to the presence of a spirotriazole core instead of the more common benzimidazole core.

Objectives: Here, a case of fatal intoxication of a long-term drug user after consumption of spirochlorphine in combination with multiple benzodiazepines is described. The young man was found dead after suffering a relapse following detoxification treatment. Spirochlorphine was quantified in post-mortem specimens collected during autopsy.

Methods: Spirochlorphine concentrations in heart blood, femoral blood, liver, kidney and stomach contents were quantified by liquid chromatography-tandem mass spectrometry (LC-MS/MS) using a standard addition method. Quantification in serum, urine and hair was performed by LC-MS/MS using external matrix calibration. *In vitro* potency of spirochlorphine at the μ-opioid receptor was assessed using a G-protein based receptor activation assay.

Results: Spirochlorphine concentrations were 4.6 ng/mL in heart blood, 9.5 ng/mL in femoral blood, 95 ng/g in kidney tissue, 64 ng/g in liver tissue and 191 ng/mL (absolute amount 57 μg) in stomach contents. A concentration of 18 ng/mL spirochlorphine was detected in post-mortem urine. In addition to spirochlorphine, other relevant findings in femoral blood included desalkylflurazepam (250 ng/mL), rilmazolam (25 ng/mL), desmethyl rilmazolam (2.9 ng/mL), di-desmethyl rilmazolam (2.9 ng/mL), diazepam (8.3 ng/mL), nordazepam (16 ng/mL), bupropion (51 ng/mL), hydroxybupropion (140 ng/mL) and levetiracetam (2,600 ng/mL). Hair analysis revealed use of various benzodiazepines and the opioid 2-methyl-AP-237 in the months prior to death.

Discussion: Spirochlorphine shows a slightly higher *in vitro* potency than fentanyl, reflected by a lower EC_{50} of 8.3 nM compared to 12.2 nM (fentanyl); thus, even low doses can be lethal or cause severe adverse effects. The detected concentrations of the benzodiazepines desalkylflurazepam and rilmazolam are considered sufficient for acute effects and therefore could have exerted a significant additive depressant effect. The concentrations of levetiracetam, nordazepam and diazepam fall within the subtherapeutic range, while bupropion and hydroxybupropion concentrations are consistent with therapeutic use. The lower concentration of spirochlorphine in heart blood compared to femoral blood might be explained by a possible dilution with pericardial fluid during sample collection. Therefore, post-mortem redistribution of the opioid as seen for

chemically similar opioids could not be assessed. In conclusion, death was attributed to a fatal mixed intoxication involving the NSO spirochlorphine and the benzodiazepines rilmazolam and desalkylflurazepam. Given the fatal outcome associated with the consumption of spirochlorphine in combination with the two benzodiazepines and considering past use of benzodiazepines with development of tolerance, a toxicological significance score of 3 was assigned to this NSO, identifying it as the cause of death.

Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

O-110

What's in My Meth? Investigating Incidental Findings in Methamphetamine-Positive Postmortem Toxicology Drug Screens

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Abstract

Introduction: Broad-scope drug screening is an initial step in toxicology testing for postmortem death investigation. Full scan drug screening by gas chromatography mass spectrometry (GC/MS) has been a reliable method utilized for many years. However, as the landscape of drugs of interest has changed, testing methods have also evolved. Liquid chromatography (LC) paired with quadrupole time-of-flight mass spectrometry (LC/QTOF-MS) is becoming an effective tool for drug screening as more drugs are amenable to LC, accurate mass measurements aid in identifying nominally similar compounds, and fragmentation may differentiate isomers.

Methamphetamine is a central nervous stimulant often abused for its mood-enhancing and euphoric effects. Illicit preparations of methamphetamine may contain impurities that can also be detected in toxicological analyses. To illustrate how LC/QTOF-MS can enhance drug screen findings, a series of postmortem methamphetamine-positive cases with incidental GC/MS findings of “mephentermine” were investigated. Mephentermine, also a stimulant, is used clinically to treat hypotension and is not often associated with methamphetamine use.

Objectives: Highlight the utility of LC/QTOF-MS for accurate identification of compounds in toxicological testing.

Methods: Blood specimens were prepared via alkaline liquid-liquid extraction with acid back extraction and analyzed on GC/MS utilizing a temperature program and 15 m VF-5MS column. MS data were acquired with electron impact ionization (70 eV) in full scan mode (m/z 40-500). Methamphetamine-positive specimens were confirmed via solid phase extraction and LC tandem mass spectrometry. Further investigations were conducted using LC/QTOF-MS with a 100 mm phenyl hexyl column. MS data were acquired in scan mode (m/z 50-550) using positive electrospray ionization; MS/MS data were acquired under information dependent conditions in scan mode (m/z 25-550).

Results: Methamphetamine was presumptively identified in 136 blood specimens using full scan GC/MS. In 29 of those cases, mephentermine was presumptively identified with mass spectral match scores ranging from 61-92 using the AAFS2004 library. Concentrations of methamphetamine confirmed in blood ranged from 0.005 to >1.0 mg/L. Mephentermine was only present in samples where methamphetamine was ≥0.14 mg/L. LC/QTOF-MS drug screening was performed for 13 of the “mephentermine” positive cases. Retrospective data review revealed that mephentermine was not present, and instead, N,N-dimethylamphetamine (DMA) was identified.

On full scan GC/MS, mephentermine and DMA share retention time and mass spectra. However, on LC/QTOF-MS, despite having identical molecular ions and similar retention times (2.5 vs 2.7 min), the MS/MS spectra differed. Mephentermine had prominent fragments of m/z 32.04, 65.03, 91.05 (base peak), 105.07, 133.10, and 164.14. DMA fragments included m/z 46.06, 91.05 (base peak), 119.08, and 164.14.

Discussion: DMA is a stimulant, N-methylated analog of methamphetamine, and isomer of mephentermine. It is often found as an impurity in illicit methamphetamine material and could be an expected finding in methamphetamine-related cases, especially if methamphetamine is found in high concentration. LC/QTOF-MS allowed for differentiation of these isomers when GC/MS was unable. Structurally related compounds should be considered when developing methods and evaluating case data. LC/QTOF-MS is one tool that can aid in differentiating similar compounds for accurate reporting of toxicology findings.

Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

O-111

Emergence of Medetomidine in San Francisco Forensic Toxicology Casework

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Abstract

Introduction: The illicit opioid landscape has undergone rapid transformation over the past decade, driven by the proliferation of fentanyl and the introduction of novel adulterants. Among these, veterinary α_2-adrenergic agonists have emerged as significant contributors to overdose risk. Xylazine was the first to become widely embedded in the fentanyl supply, with increasing detections across the United States, including a marked rise in San Francisco beginning in 2022. More recently, medetomidine has been identified in eastern and midwestern jurisdictions; however, data describing its presence in western U.S. forensic casework remain limited. Given its high potency and lack of reversal by naloxone, medetomidine represents a critical emerging concern.

Objectives: This study aimed to characterize medetomidine detections in postmortem casework from a western U.S. medical examiner jurisdiction during 2025, including demographic, circumstantial, and toxicological features, and to contextualize these findings within the evolving landscape of veterinary sedative adulteration.

Methods: A retrospective review of all postmortem toxicology cases analyzed in 2025 was conducted. Cases were included if medetomidine was detected in any biological specimen. Toxicological analysis employed a comprehensive workflow incorporating qualitative confirmation by liquid chromatography quadrupole time-of-flight mass spectrometry (LC-QTOF-MS) with a reporting limit of 0.10 ng/mL in both blood and urine, and targeted quantitation by standard addition when applicable. Data collected included demographics, manner and cause of death, specimen type, medetomidine concentrations, and co-detected substances. Results were summarized descriptively due to the limited number of cases.

Results: Medetomidine was detected in eight postmortem cases, all classified as accidental deaths. Decedents ranged in age from 30 to 75 years and were predominantly male. Medetomidine was included in the cause of death in six cases, typically in the context of mixed-drug intoxication. All cases involved fentanyl co-detection. Stimulants were present in seven cases, most commonly methamphetamine (6/8), followed by cocaine (4/8). Additional sedatives included novel benzodiazepines (3/8) and xylazine (3/8), with one case involving kavain. Blood concentrations were frequently low or near reporting thresholds (0.10-0.34 ng/mL), and one case demonstrated urine-only detection (4.2 ng/mL). Following the addition of 3-hydroxymedetomidine to the analytical library, retrospective review identified this metabolite in the urine of three of the eight cases. Death circumstances were consistent with opioid-related fatalities, and naloxone administration was reported in at least one case without apparent effect.

Discussion: These findings demonstrate that medetomidine has emerged in postmortem forensic casework in a western U.S. jurisdiction and can contribute to fatal overdose in polysubstance settings. Its appearance coincides with a decline in xylazine detections in 2025, suggesting a potential shift in veterinary sedative adulteration within the illicit fentanyl supply. Interpretation is complicated by low blood concentrations, pharmacokinetics, and polysubstance exposure, emphasizing the need for context-driven forensic evaluation. Routine inclusion of medetomidine in toxicology testing panels and continued surveillance will be essential for accurate cause-of-death certification and monitoring of the evolving drug supply.

Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

O-112

Kratom-Related Fatalities With Opioid Co-Detection: Toxicological Complexity and Medico-Legal Challenges

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Abstract

Introduction: Deaths involving kratom remain challenging to interpret in forensic practice because mitragynine is often detected in complex polydrug settings. Among the substances most likely to complicate causal assessment, opioids are particularly relevant because of their well-established lethality and their frequent co-detection in postmortem cases involving kratom.

Objectives: This study aimed to characterize kratom-related fatalities collected from multiple U.S. forensic institutions, with particular focus on cases in which mitragynine was detected together with opioids. The study assessed the prevalence of opioid co-detection, the main autopsy findings, reported mitragynine and opioid concentrations, and the certified cause of death.

Methods: A total of 215 autopsy cases involving postmortem mitragynine identification between 2013 and 2024 were systematically reviewed. Extracted data included mitragynine concentrations, co-detected substances, analytical methods, toxicological completeness, demographic data, autopsy findings, and the certified causes of death. For the purposes of this study, full toxicological testing was defined as broad toxicological screening followed by analytical confirmation and, when available, quantitation of all relevant detected substances. The overall series was analyzed descriptively, with focused evaluation of opioid-positive cases and their toxicological and medico-legal characteristics.

Results: Among 215 kratom-positive fatalities, 114 (53.0%) had co-detected opioids. In this subgroup, the median age was 35 years (IQR 32-41), and most decedents were male (87/114, 76.3%). Mitragynine concentrations were quantified in 110 cases, with a median value of 0.1545 mg/L (range 0.0020-7.223 mg/L). Fentanyl or fentanyl-related compounds were identified in 91.2% (104/114) of cases, showing that the opioid-positive subgroup was predominantly associated with synthetic opioids. In 102 out of 114 cases (89.5%), opioids were accompanied by at least one additional non-opioid drug class, indicating a predominantly polydrug pattern. The most frequently co-detected non-opioid classes were stimulants (49.1%), alcohol (31.6%), other non-opioid drugs (31.6%), benzodiazepines (29.8%), and cannabinoids (25.4%). In 105 out of 114 cases (92.1%), the cause of death explicitly referred to opioids. However, 4 cases (3.5%) were certified as mitragynine intoxication alone, without mention of opioids despite toxicological opioids co-detection. Full toxicological testing was documented in only 66 of the 114 opioid-positive cases (57.9%).

Discussion: Opioid co-detection, predominantly involving fentanyl and related compounds, was the most common pattern in kratom-positive fatalities, indicating that these deaths typically occur in a high-risk polydrug setting rather than in isolation. The finding that some opioid-positive cases were nevertheless certified as due to mitragynine alone highlights the inherent complexity of cause-of-death assessment in these investigations. As full toxicological testing was only documented for part of this subgroup, any interpretation of the role of mitragynine should be made with caution and within the appropriate toxicological context. These findings highlight the importance of standardised toxicological protocols and consistent cause-of-death certification practices in cases involving kratom and opioids.

Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

O-113

Variability in Cause of Death Attribution in Nitazene-Related Fatalities

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Abstract

Introduction: Nitazenes are highly potent 2-benzylbenzimidazole opioids increasingly detected in drug-related deaths. Professional organizations advocate for the inclusion of all detected substances contributing to death in the cause of death (COD) statement; however, with increasing emergence of novel substances in the drug supply, it is unclear how uniformly this approach is applied.

Objectives: We characterized nitazene-involved overdose deaths and examined inter-rater agreement in nitazene COD attribution.

Methods: We conducted a retrospective cohort study of overdose deaths between October 1, 2019, and December 31, 2022, in Ontario, Canada, using data from the Centre of Forensic Sciences. We included cases if at least one nitazene was detected in post-mortem blood samples and COD was attributed to drug toxicity. Each case was reviewed by an independent forensic pathologist who was blinded to the interpretation of the original pathologist. The primary outcome was agreement between pathologist COD statements as it relates to the inclusion of nitazenes, calculated using percent agreement and Cohen's kappa. Secondary analyses examined factors associated with agreement.

Results: We identified 86 cases that met inclusion criteria. Most cases (n=84; 97.7%) were associated with another substance (median: 7 substances; IQR 6-10 substances). The most frequently co-detected substance was fentanyl (n=69; 80.2%), with a median concentration of 31 ng/ml (IQR 11.5-40.0 ng/ml). Novel psychoactive substances were detected in 71 (82.6%) cases and multiple nitazenes were detected in 9 (10.5%) cases.

Nitazenes were incorporated into COD statements in 23 cases by the original pathologist (13 distinct pathologists) with no agreement between pathologists as to whether nitazenes were incorporated (percent agreement 25.6%; κ = -0.02). In contrast, fentanyl was included in the COD statement in 66 cases (95.7% of fentanyl-detected cases), even when present below the lower reporting limit, with moderate agreement between pathologists (percent agreement 97.1%, κ = 0.49). Overall, among nitazene-involved cases, factors associated with agreement included fentanyl concentration below the lower reporting limit (<1.3ng/ml; p=0.015) and quantified or "detected" rather than "unconfirmed" nitazene status on toxicology report (RR 0.31, 95% CI 0.16–0.58; p=0.002). However, among quantified or "detected" nitazene cases, 40% (n=6) were not included in the COD by the original case pathologist. Concordant cases had significantly fewer co-detected substances than discordant cases (median 5, IQR 4-6 vs median 8, IQR 7-10; p<0.001). Concordance was higher in 2022 than 2021 (36.6% vs 15.4%; RR 2.38, 95% CI 1.03-5.50), with original pathologist attribution increasing from 15.4% to 39.0%.

Discussion: Overdose deaths with nitazenes generally involved multiple substances, with no agreement between pathologists as to whether nitazenes contributed to death. Fentanyl was the most commonly co-detected substance and was consistently incorporated into COD statements, while nitazenes were not. Our findings suggest that contribution of emerging substances, like nitazenes, to overdose deaths is underestimated, especially as they first appear in the drug supply, with meaningful public health consequences for those relying on COD statement data for surveillance. Further analysis is underway exploring current trends and the written opinions proffered in tandem with COD statements.

Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

O-114

Synthetic Cannabinoid Symptomology in North Carolina Deaths

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Abstract

Introduction: Newly emerging synthetic cannabinoids are structurally diverse, under-studied, and much remains unknown about their toxicity mechanisms and relative lethality. Consumption is reported to result in a wide variety of symptoms including seizures, respiratory depression, vomiting, heart arrhythmias, and kidney damage. Due to this, pathologists vary widely in their willingness to ascribe death to these compounds and/or recognize symptomology as possibly indicative of synthetic cannabinoid use.

Objectives: This presentation will share an overview of what is known about synthetic cannabinoids in the human body and possible toxicity mechanisms. It will share aggregate data about clinical presentations of synthetic cannabinoid overdose deaths in both prison and non-prison settings. A few detailed case studies will be shared, with an analysis of symptomology and co-morbidities in relation to known synthetic cannabinoid effects.

Methods: Specimens were screened for common and novel drugs via a targeted Liquid Chromatography/Mass Spectrometry method. Further investigation in the form of targeted analysis for synthetic cannabinoids or re-interrogation of initial screen data was conducted if scene evidence was indicative of possible synthetic cannabinoid consumption, or alternately if no sufficient anatomical or drug cause of death existed. Cases positive for synthetic cannabinoids were qualitatively confirmed, and information from Toxicology Request Forms, Case Calls, Autopsy Reports and Reports of Investigation by Medical Examiner were combined as available. Case characteristics of interest were then extracted and counted.

Results: As of April 1, 2026, the North Carolina Office of the Chief Medical Examiner has detected synthetic cannabinoids in 91 cases (54 in prisons and 37 outside of prisons) from 2020- March 2026, including MDMB-4en-PINACA and/or metabolite (52 detections), 5F-MDMB-PICA and/or metabolite (22), 5F-ADB and/or metabolite (19), ADB-BUTINACA (9), 4F-MDMB-BUTINACA and/or metabolite (8), and one each of 4-cyano CUMYL-BUTINACA and/or metabolite, AB-FUBINACA, and AMB-FUBINACA. Of these cases, death has been at least partially attributed to synthetic cannabinoids by the pathologist in 52 cases (13 cases pending COD). Twenty-three cases mentioned additional heart disease in the cause of death, nine cases presented with documented vomiting or vomit present, and three cases had documented seizures. Of the heart disease cases, four also included vomiting, and five cases mentioned the possibility of cardiac arrhythmia. Eight cases simply listed a decedent “found down” with a cause of death listed as synthetic cannabinoid toxicity- of these, one specifically mentioned bystanders attesting to “strange noises, maybe breathing, that didn’t sound right”. Other potentially lethal drugs were more likely to be present outside of the prison setting, and of the 20 cases where other drugs (e.g. fentanyl, cocaine, methamphetamine) were present, eight cases included the synthetic cannabinoid on the final cause of death and 12 did not.

Discussion: Attribution of death to synthetic cannabinoid poisoning is complicated by their complex and sometimes contradictory symptomology, relatively small case numbers, and frequent lack of detailed clinical documentation. Pathologists and toxicologists should be aware

of possible symptomology of vomiting, seizures, sudden collapse due to cardiac arrhythmias, respiratory depression, and kidney injury. Death attribution will be more likely in the absence of other competing causes of death due to the complexity of these cases.

Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

O-115

Hidden in Plain Sight: Lessons From NPS Fatalities in Luxembourg

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Abstract

Introduction: Fatalities associated with novel psychoactive substances (NPS) have increased markedly in recent years, driven in part by the emergence of highly potent and toxic compound classes such as synthetic opioids. While NPS-related cases remain rare in Luxembourg, their identification in forensic autopsies is particularly challenging, especially in the absence of background information or relevant findings at the death scene. In Luxembourg, all forensic autopsies systematically undergo comprehensive toxicological analysis, providing a unique opportunity to detect otherwise unrecognized substance involvement. This approach enabled the identification of the first NPS-related fatalities in the country in 2025.

Objectives: The objective is to demonstrate the value of systematic toxicological analysis in forensic autopsies through three cases of NPS-related deaths, including one case with no prior suspicion of drug use and one representing the first reported fatality associated with 2-fluoromethamphetamine.

Methods: Routine toxicological analysis was performed and includes liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS) screening of blood and gas chromatography–mass spectrometry (GC-MS) screening of urine and gastric content. In all cases, peripheral and cardiac blood, urine, and gastric content were available and analyzed. Peripheral blood samples were additionally submitted to an external laboratory for NPS confirmation and quantification.

Results: Case 1 involved an individual with a history of drug use and psychiatric disorders, found deceased at home. Multiple unlabeled pills and powders were found near the body. Toxicological analyses revealed high concentrations of the NPS 2-fluoromethamphetamine and bromazolam in peripheral blood, alongside conventional drugs, including therapeutic concentrations of escitalopram and desmethyltramadol, as well as low concentrations of trazodone, bupropion, and levomepromazine. This case represents the first reported fatality associated with 2-fluoromethamphetamine.

Case 2 concerned an individual with known alcohol abuse but no documented drug use, found deceased at home. Scene findings included empty alcohol bottles and three bags containing powders, with labels for 2F-DCK (2-Fluoro-Deschloroketamine), methiodone and 1cP-LSD. Toxicological results in blood identified alcohol, tramadol, alprazolam, mitragynine and confirmed the NPS 2F-DCK and methiodone, while 1cP-LSD was not found. This case highlights complex patterns of combined substance use.

Case 3 involved an individual with no known history of drug use or preexisting conditions, found deceased at home. Toxicological analyses in blood revealed high concentrations of deschloroketamine together with desalkylflurazepam and desalkylgidazepam. In this case, toxicological findings were unexpected and decisive in establishing the cause of death in an otherwise unexplained fatality.

Discussion: In all three cases, death was attributed to fatal polyintoxication involving multiple NPS. While investigative findings in cases 1 and 2 suggested possible substance use, case 3 illustrates the critical role of comprehensive toxicological analysis in uncovering otherwise hidden causes of death. These findings highlight the risk of failing to recognize NPS involvement in the absence of systematic toxicological analysis and underscore the importance of forensic toxicology in cause-of-death determinations. The presented cases further demonstrate the increasing diversity of substances and patterns of polyintoxication in NPS-related fatalities and the need for continuous adaptation of analytical strategies.

Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

O-116

Where Did the Blue Intestine Come From? A Case Report of a Fatal Combined Intoxication With Cocaine, γ-Hydroxybutyric Acid (GHB), Morphine, and Rilmazolam

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Abstract

Introduction: A 30-year-old male person was found dead in July, 2024. During police investigation the girlfriend reported that the deceased man took different drugs almost daily. Additionally he drank alcohol and smoked cigarettes daily. In the surrounding area of the deceased the police found capsules of morphine, a transparent bottle labeled with 1,4-BDO containing a colorless liquid, a cup with an unknown crystalline powder, and a brown bottle with the label ODMT containing turquoise powder. During forensic autopsy the coroner mentioned, in addition to unspecific signs of intoxication, a partially bright turquoise blue colored small intestine.

Methods: Blood and urine samples were analyzed using head space-gas chromatography coupled with flame ionization detection (HS-GC-FID) for blood and urine alcohol analysis. Gas chromatography coupled with mass spectrometry (GC-MS) and liquid chromatography coupled to a quadropoly time-of-flight mass spectrometry (LC-QToF-MS) analysis were used after solid phase extraction on a mixed mode cartridge (C18Ar/Mp3) for analysis of drugs and other xenobiotics. Additionally, the turquoise powder was analyzed in the laboratory of the State Office of Criminal Investigation Brandenburg using liquid chromatography mass spectrometry (LC-MS^n, ToxTyper®) after liquid-liquid-extraction using chloroform/methanol (v:v, 1:1). Finally, blood and urine samples were send to the specialized forensic laboratory of the Institute of Legal Medicine, Freiburg, Germany for quantification of rilmazolam and its metabolites.

Results: Blood and urine alcohol analysis were negative. Analysis of femoral blood samples yielded the following results: cocaine: 0.091 µg/mL, benzoylecgonine: 0.440 µg/mL, methylecgonine: 0.140 µg/mL, γ-hydroxybutyric acid (GHB): 31 µg/mL, and morphine: 0.440 µg/mL. Analysis for o-desmethyltramadol (ODMT) was negative in blood and urine. In-house analysis of the turquoise powder showed traces of ODMT and an unknown substance. Identification as rilmazolam was performed by the laboratory of the State Office of Criminal Investigation Brandenburg. Rilmazolam and its metabolites in femoral blood were quantified by laboratory of the Institute of Legal Medicine, Freiburg with concentrations of rilmazolam: 0.0023 µg/mL, desmethyl-rilmazolam: approximately 0.026 µg/mL, and di-desmethyl-rilmazolam: approximately 0.024 µg/mL.

Discussion: Beside the toxic respectively potentially lethal concentrations of cocaine and its metabolites, GHB, and morphine in femoral blood, the turquoise small intestine and sized powder showed the presence of an additional unknown compound. Online research led to vendor-pages selling bright blue up to turquoise-colored tablets containing rilmazolam. An oral ingestion of those kind of tablets could explain the blue coloring. Even if the combination of cocaine, GHB, and morphine was able to explain the death of the young male, the detection of the new designer benzodiazepine rilmazolam was important for further investigations especially in other cases.

Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

O-117

Potential Fatal Interaction Between Psilocin, Phosphodiesterase Inhibition, and Adenosine Antagonism: A Case Report and Mechanistic Hypothesis

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Abstract

Introduction: Psilocybin-containing mushrooms are increasingly used in both recreational and therapeutic contexts and are generally regarded as having low acute toxicity. However, psilocin, the active metabolite, has been shown to exert cardiostimulatory effects via serotonin receptor pathways including some evidence that cardiac 5-HT4 receptors may enhance chronotropy and inotropy through cyclic AMP (cAMP) dependent mechanisms. The presence of co-exposures that either increase intracellular cAMP or remove endogenous inhibitory controls such as phosphodiesterase (PDE) inhibitors and adenosine receptor antagonists may potentiate these effects and alter cardiac electrophysiology.

Objectives: To describe a fatal case involving psilocin and co-exposure to caffeine, theobromine, and acyclovir in the context of an otherwise unremarkable medical history and post-mortem examination, and to propose a novel multi-mechanistic drug interaction as a plausible cause of death.

Methods: A full post-mortem examination, including specialist cardiac assessment, was undertaken. Toxicological analysis of post-mortem blood and urine followed a validated forensic workflow, comprising headspace gas chromatography for ethanol and other volatile substances, and broad-spectrum drug screening by liquid chromatography-high-resolution mass spectrometry. Detected xenobiotics were quantified using an internally validated standard-addition approach, employed because the analytes of interest were not included within routine validated quantitative assays. The standard-addition method was validated using local protocols with assessment of calibration performance and uncertainty from the standard-addition regression.

Results: A 34-year-old female with no significant medical history was found deceased following ingestion of a decoction containing psilocybin-containing mushrooms and high-cacao chocolate. There was no known history of substance misuse. Post-mortem examination, including detailed cardiac assessment, was unremarkable, with no structural abnormalities identified to account for death.

Toxicological analysis identified psilocin at concentrations consistent with recreational use. Caffeine and theobromine were at concentrations consistent with chocolate and caffeinated beverage consumption. Acyclovir was also identified at therapeutic concentrations. No other drugs or toxins of significance were detected.

Discussion: Psilocin is a potent agonist at 5-HT2A receptors but has also been shown to activate cardiac 5-HT4 receptors, leading to increased cAMP-mediated signalling within cardiac myocytes. Methylxanthines such as caffeine and theobromine act as non-selective PDE inhibitors, reducing cAMP breakdown, and as adenosine receptor antagonists, removing inhibitory modulation of cardiac excitability. These dual actions may significantly enhance intracellular signalling pathways associated with increased heart rate and contractility. The detection of acyclovir, for which emerging evidence suggests possible PDE-inhibitory effects, may represent an additional contributing factor.

We propose that the combination of psilocin-mediated stimulation, PDE inhibition, and adenosine receptor blockade may result in a synergistic increase in cardiac excitability. This convergence of mechanisms may predispose to fatal arrhythmia, particularly in the absence of structural cardiac disease.

While causality cannot be definitively established, the absence of anatomical findings and the presence of pharmacodynamic influences support a plausible functional arrhythmic mechanism. This case highlights a potentially underrecognized interaction between psilocybin-containing mushrooms, dietary methylxanthines, and commonly prescribed medications. As patterns of psychedelic use evolve, particularly in combination with chocolate products, such interactions may become more relevant. Further investigation into these combined effects is warranted.

Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

O-118

A Data Processing Framework for the Untargeted Analysis of High-Resolution Mass Spectrometry Data to Enable NPS Detection and Metabolomics in Forensic Casework

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Abstract

Introduction: The development of untargeted acquisition techniques has been a promising step towards a general untargeted screening (GUS) method for forensic toxicology. However, the processing methods for this data are currently targeted, limiting the detections to already characterized substances and resulting in significant resources to process acquired untargeted data.

Objectives: The purpose of this study was to create a GUS method that is scalable for routine casework, with the capability to elucidate molecular structure directly from spectral data to enhance the detection of novel psychoactive substance (NPS). The formation of an untargeted processing method unlocks the potential for metabolomics studies for forensic predictions such as postmortem interval (PMI) and crash-phase methamphetamine studies. This method is intended for routine implementation within the San Francisco Office of the Chief Medical Examiner (OCME) routine casework workflow.

Methods: A pipeline that utilizes Python and several spectral processing programs was developed to characterize the untargeted acquisition small molecules within scope within a casework sample. Proteowizard was applied to vendor centroid samples from raw acquisition batch files. A MZMine workflow was developed to match the acquisition parameters and was validated against authentic confirmed detections from routine casework received in 2025.

The internal standards and confirmed reported detections were identified through optimized 5 ppm and 15 ppm mass error thresholds and 100% and 10% retention time thresholds to prevent re-interrogation of known substances. The blank matrix of each batch was used to filter endogenous substances through mass error, relative retention time (RRT) error, and area comparisons. Feature's tandem mass (MS/MS) spectrum and MS spectrum were plotted programmatically for evaluation.

Results: The pipeline analyzed ~4,000,000 unique small molecules acquired in 276 cases across 11 routine batches and achieved a 96% match rate across 1344 forensic toxicologist confirmed detections, with a manual inspection of peaks and fragments further confirming chromatographic matches. The mean RRT for matches was 11% and the mean mass error was 1 ppm. A scoring criterion was developed to evaluate unmatched peak detections, with 83% of unmatched detections receiving sub-optimal scores making these peaks difficult detections for an automated pipeline.

Discussion: A true GUS method through DL holds incredible promise to enhance routine screening methods. Machine learning techniques can parse through millions of small molecules.

In this study, the developed approach demonstrated robust performance in accurately identifying within-scope components, supporting its utility for routine forensic applications. An untargeted analysis can serve as an early detection system for the discovery of NPS, with the goal of incorporation into reference libraries to enhance routine screening. Metabolomics studies can fully leverage untargeted datasets to generate predictive, forensically relevant insights that enhance routine analytical workflows.

Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

O-119

Interpretive Toxicology: Fitting Probability Distributions to Post-Mortem Drug Case Data

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Abstract

Introduction: There have been loud calls around the world to improve the robustness of forensic science interpretive practices, leading to the publication of an International Standard for the interpretation of forensic evidence (ISO 21043-4). Post-mortem forensic toxicology has seen limited progress in this regard. However recent work has proposed an interpretive model that provides a transparent, probabilistic approach to decision-making on the role of a drug in the cause of death. This occurs through the evaluation of likelihood ratios by the forensic toxicologist, and of prior/posterior probabilities by the fact finder. The use of this approach requires the estimation of the probability distribution of femoral blood drug concentrations. To date, the most appropriate methodology to model this has not been determined.

Objectives: To determine if post-mortem femoral blood concentrations are best modelled by non-parametric modelling (kernel density estimation (KDE)) or parametric modelling with known distributions (such as Log-Normal).

To determine which underlying probability distributions best describe post-mortem femoral blood drug concentrations.

Methods: To compare KDE with parametric distribution fitting under controlled conditions, simulated datasets (n = 5–200) were generated in R from a Log-Normal distribution. Each dataset was fitted using KDE (R package ks) and a Log-Normal distribution (R package GAMLSS). The goodness-of-fit was assessed using Kullback–Leibler (KL) divergence. To understand which probability distributions best describe post-mortem femoral blood drug concentrations, data from routine toxicology for 47 different drugs and metabolites were used. Sample sizes were >20 cases for each drug. We considered right-skewed continuous distributions supported on (0, ∞). These were Log-Normal, inverse Gaussian, Gamma, Generalised Gamma, Weibull, and Normal (Gaussian) distribution. The probability distribution that best fit the data was identified as the one with the lowest Akaike Information Criterion (AIC). Again, the GAMLSS package in R was used to fit probability distributions, this time accounting for left censoring below the limit of quantification (LOQ) by using left-censored probability density fitting.

Results: Results of the analyses showed that the parametric approach, using a known distribution, allowed better fitting of the underlying data with fewer cases (KL divergence ~0.07 at 20 cases) than would KDE (KL divergence ~0.32 at 20 cases). The second part of the study showed that post-mortem femoral blood drug data are best described by either Log-Normal or Inverse-Gaussian distributions (~64% of best-fit cases); however, all distributions used were represented, except the Normal distribution.

Discussion: These results suggest that the best approach for modelling the probability distributions of post-mortem femoral blood drug data is to use known probability distributions, that are right-skewed and are continuous from (0, ∞). Left censoring should be incorporated into the modelling framework to account for observations below the LOQ. The use of multiple, known distributions allows the best opportunity for an optimal fit of a probability distribution for the data. This approach gives a robust framework for fitting post-mortem femoral blood drug concentration data. This will be necessary to implement an interpretive approach to the role of drugs in the cause of death.

Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

O-120

Don’t Let AI Think for You; Make AI Work for You

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Abstract

Introduction: The integration of Artificial Intelligence (AI) in forensic science is frequently met with skepticism, primarily due to concerns regarding the "black box" nature of algorithmic decision-making in casework. However, a significant yet underutilized application of AI lies in its ability to serve as a sophisticated software developer for custom laboratory tooling. Rather than delegating forensic conclusions to an AI agent, curious scientists can utilize Large Language Models (LLMs) to bridge the gap between bench chemistry and software engineering. This approach allows anyone with detailed workflow knowledge to develop efficient solutions in modern programming languages, regardless of their formal computer science background.

Objectives: This presentation aims to demonstrate a paradigm shift: viewing AI as a tool-building assistant rather than an automated analyst. The primary objective is to illustrate how AI can be utilized to build modern software from scratch for repetitive data-handling and database querying tasks, at no additional cost.

Methods: The methodology follows a four-stage lifecycle: Workflow Analysis, AI-Assisted Design, Unit Testing, and Deployment. Routine laboratory tasks from the analyst to management level were assessed for improvements, including manual spreadsheet tracking, raw instrument data PDF binding, and ad-hoc database queries. The constraints and details of each situation were presented to an AI agent to generate modular code in Python and Go. The resulting code underwent traditional software validation—unit testing and comparison against manual outputs—to ensure that the tool performed predictably and accurately within the laboratory's secure local network environment.

Results: The application of AI-assisted custom tools resulted in a quantifiable reduction in case processing time, a decrease in transcription errors, and improved management oversight. The automation of PDF binding and renaming transformed a multi-step manual process into a single-click operation, ensuring consistency across all case files. The deployment of a local web dashboard provided real-time visibility into laboratory metrics and case statuses without the need for expensive proprietary software. Weekly automated reports inform both management and clients of case processing bottlenecks. By using AI to write the underlying framework, the development time for these utilities was reduced from months to days, allowing for rapid iteration and deployment of solutions tailored to specific departmental needs.

Discussion: The true utility of AI in forensics lies in its ability to empower the innovation of competent, knowledgeable scientists. Rather than delegating judgment, LLMs can be used to dismantle technical barriers and solve operational problems that were previously resource restricted. This tool-builder approach effectively neutralizes the black-box concerns often associated with AI; because the scientist oversees the development of the logic, they can review, test, and maintain full ownership of the source code. The next generation of forensic leadership must be technologically proficient, utilizing AI to architect the infrastructure of the modern laboratory. This paradigm ensures the human expert remains the sole arbiter of forensic results, supported by a robust framework of custom-built, validated automation.

Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

O-121

Leveraging Open-Source Computational Tools to Generate Contrived Data for Validating Data Processing Systems

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Abstract

Introduction: Proprietary laboratory information management systems (LIMS) and mass spectrometry data processing platforms occupy a central role in clinical toxicology workflows, yet their internal processing logic — peak integration algorithms, calibration weighting schemes, and rule-based quality control (QC) flagging — is largely opaque to end users. This opacity creates a meaningful gap: laboratories operating under regulatory oversight cannot independently verify that software behavior conforms to its specified rule structure, nor can they retrospectively interrogate processed batches when software updates or configuration changes occur. The emergence of accessible scientific computing tools offers a path toward transparent, auditable replication of these workflows.

Objectives: To develop an open-source computational pipeline that faithfully replicates the quantitative output of a predicate commercial LC-MS/MS data processing platform for urine THCA by liquid chromatography-tandem mass spectrometry (LC-MS/MS), and to establish this pipeline as a practical instrument for prospective rule-structure validation and retrospective audit of production sample results.

Methods: Raw chromatographic data were extracted from the proprietary platform's batch export format (base64-encoded float64 chromatogram arrays embedded in structured JSON). Peak integration was implemented using scipy.signal.peak_widths at a relative height threshold of 0.99, which demonstrated superior handling of intra-peak dip artifacts compared to boundary-walking approaches. Calibration was performed using $1/x^2$ weighted linear regression (LLOQ 5 ng/mL, ULOQ 1000 ng/mL, origin ignored) over five calibrator levels. Ion ratio QC was calculated for both the THCCOOH analyte (QUANT 345.2→299.2; QUAL 345.2→193.1) and the deuterated internal standard THCCOOH-D3 (ISTD QUANT 348.2→302.2; ISTD QUAL 348.2→260.2), with signed percent bias computed against calibrator-derived means and evaluated against ±20%/±30% acceptance thresholds. A synthetic sample generator was developed to produce controlled test cases with independently specified ion-ratio biases for stress-testing QC rule boundaries. Method agreement against the predicate platform was assessed by Passing-Bablok regression.

Results: Across a validation cohort of patient samples and QC materials, the pipeline achieved a Passing-Bablok slope of 1.006 (95% CI approaching unity), mean proportional bias of −0.31%, and all individual sample results within ±1.9% of predicate-reported values — well within accepted method comparison criteria. Ion ratio QC flags showed complete concordance with predicate determinations. Ongoing work will extend validation to larger archived batch sets spanning multiple instruments and reagent lot configurations, using the pipeline as an independent audit tool to confirm that the commercial platform's applied rule structure is consistent with its configured parameters across the production sample history.

Discussion: These results demonstrate that a transparent, Python-based pipeline can achieve near-exact replication of a commercial LC-MS/MS quantitation workflow for THCCOOH. Beyond serving as a method comparison tool, this approach offers a novel framework for prospective rule-structure validation — enabling laboratories to confirm that algorithmic behavior in production software matches its specified configuration before and after software changes. Retrospective application to released production batches will provide an additional layer of assurance relevant to laboratory accreditation and regulatory compliance. The underlying architecture is generalizable to other MRM-based clinical assays.

Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

O-122

High-Resolution Mass Spectrometry-Based Molecular Networking Workflow for Untargeted Detection of Emerging Nitazenes in Seized Materials

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Abstract

Introduction: The continuous emergence of New Psychoactive Substances poses a significant analytical challenge for forensic and toxicological laboratories. Among these, nitazenes have gained increasing attention due to their high potency and extensive structural diversity. This variability complicates their identification, particularly as newly emerging analogues are often encountered before analytical reference standards become available. Therefore, untargeted analytical approaches have become essential in forensic toxicology. However, liquid chromatography coupled to high-resolution mass spectrometry (LC-HRMS) data are complex and an effective interpretation requires advanced data processing strategies. Molecular networking (MN) organizes MS/MS spectra based on spectral similarity, grouping structurally related compounds into clusters. This approach enables the visualization of chemical relationships within complex datasets and supports both class-based annotation and the detection of structurally related unknown compounds.

Objectives: This study presents a combined workflow for the detection of nitazenes in seized drug materials using LC-HRMS and a MN–based strategy to classify molecular features according to structural characteristics. This workflow was further applied to drug samples seized by the Sao Paulo State Police in the Region of Campinas, Brazil.

Methods: After LC reversed phase separation, MS/MS fragmentation data were acquired using an Orbitrap Exploris 120 system from thirty-seven analytical reference materials representing several nitazene structural motifs, including compounds with different core and halogen substitutions. These data were used to construct a reference-based molecular network in Compound Discoverer (v3.4) based on spectral similarity. The MN parameters were systematically optimized, considering spectral similarity score, spectral coverage, and the minimum number of shared fragment ions between MS/MS spectra. Subsequently, seventeen seized samples were analysed under identical experimental conditions and mapped onto the generated molecular network. Unknown nodes associated with the nitazene cluster were further investigated as potential novel analogues through detailed MS/MS fragmentation analysis, followed by peak isolation using preparative liquid chromatography with photodiode array detection and structural confirmation by nuclear magnetic resonance (NMR) spectroscopy.

Results: The nitazene standards were used to construct the molecular network. After MN parameters optimization, all compounds were successfully grouped into a single molecular cluster, demonstrating a grouping effectiveness despite the structural diversity of nitazenes. Cluster connectivity and cosine similarity scores indicated a stronger structural relationship among compounds sharing common core scaffolds when compared to less related nitazenes. When applied to seized materials, the workflow enabled the detection of a previously unreported nitazene in three herbal samples. These samples exhibited a metonitazene-associated unknown node with the molecular formula $C_{21}H_{25}ClN_4O_3$ and a fragmentation pattern consistent with a typical nitazene. Following peak isolation and purification, chlorometonitazene was fully characterized by LC-HRMS and NMR, representing its first identification worldwide.

Discussion: The proposed MN workflow proved effective for organizing and identifying structurally diverse nitazenes in both reference standards and seized samples. By enabling class-based annotation and the detection of structurally related unknowns, the approach facilitated the identification and full structural characterization of chlorometonitazene, a new nitazene identified for the first time, in the absence of reference standards. These findings highlight the potential of MN as a novel tool for the monitoring of emerging NPS.

Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

O-123

Behavioral Patterns and Social Media Explorations for Toxicology using Neurosymbolic AI Approaches: A Case Study for Drug Misuse

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Abstract

Introduction: Behavioral science is central to understanding how human behavioral patterns emerge and are expressed within psychological contexts. A formal ontology of behavioral patterns is therefore essential, as standardized vocabularies enable researchers to operate within a consistent, interoperable framework.

Objectives: A general and comprehensive ontology for representing behavioral learning and conditioning processes is still lacking. To address this gap, we introduce the Behavioral Patterns Ontology (BPO), a foundational framework for formally representing psychological concepts through patterns of behavior, with particular emphasis on the learning and conditioning mechanisms that shape, reinforce, and modify behavior over time. Through this analysis we aim to help identify trends, and assist toxicologists in better detecting unknown test results should new substances arise.

Methods: The current version of BPO comprises 107 classes and 12 object properties, organized through 106 hierarchical relationships and specified by 341 axioms. Together, these components provide a structured representation of behavioral processes and their interdependencies. To demonstrate its practical utility, we applied BPO to the annotation of TikTok video data and integrated the resulting semantic annotations into a Social Media Analysis Dashboard for visualization and interpretation at the individual-video level. Social media platform posts were analyzed by domain experts and annotated by computer scientists with the help of our psychology team. The data have been verified through domain expert knowledge. We further evaluated the ontology using SPARQL queries driven by the ontology across multiple use cases and have accuracy measures in place for each analysis.

Results: The results show that BPO effectively captures learning mechanisms, substance-specific behavioral patterns, and the drivers and contextual settings associated with drug-seeking behavior on TikTok. These findings suggest that BPO offers a promising foundation for the formal, reusable, and computationally tractable representation of human behavioral patterns across digital and psychological research settings.

Discussion: Although existing ontologies in the behavioral and social sciences provide important domain-specific frameworks, including models of addiction, behavior change interventions, mental functioning, emotions, and social entities, they are not designed to systematically capture the cross-context mechanisms underlying behavioral formation and adaptation. BPO addresses this unmet need by providing a transferable, semantically grounded framework for representing behavioral processes across multiple application domains.

Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

O-124

Development and Validation of a Deep-Learning Screening Method for NPS in Routine Forensic Casework

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Abstract

Introduction: Novel psychoactive substances (NPS) pose significant challenges for forensic laboratories, as the grey market can fluctuate faster than reference libraries can be updated. An untargeted processing method enables a deep learning (DL) framework capable of classifying NPS and elucidating potential structures. This can serve as an early warning system for NPS and enhance routine screening methods when applied to routine forensic casework.

Objectives: The purpose of this study was to create a general unknown screening (GUS) method that is scalable for routine casework, with the capability to elucidate molecular structure directly from spectral data to enhance the detection of NPS. This method is intended for routine implementation within the San Francisco Office of the Chief Medical Examiner (OCME) routine casework workflow.

Methods: A pipeline that utilizes Python, R, LaTeX, and spectral processing programs was developed to characterize all small molecules as features within a casework sample. Features were tokenized through cosine similarity comparisons to reference compounds and through diagnostic characteristics. A multi-layer perceptron DL neural network was trained through cross-nested validation to classify between candidate NPS and features. The DL method DarkNPS was retrained on OCME confirmed detections and used to elucidate structure.

Classified candidates were visualized and compiled into reports using LaTeX for seamless integration into the OCME Laboratory Information Management System (LIMS). A dedicated interface was developed to monitor recurring detections, identify detections consistently co-occurring with compounds within the OCME routine testing scope, and generate consensus spectra across observations.

Results: The pipeline analyzed 140 batches and 3408 cases, accumulating to ~49,000,000 unique small molecules. The classifier was trained using the NVIDIA RTX 4500 Ada Generation and evaluated on ~100,000 casework NPS across 11 drug classes and ~200,000 non-NPS small molecules. Preliminary results demonstrate a classifier detection rate of 77% with a 5% false detection rate, with an adjustable threshold designed to minimize forensic toxicologist's active screening time to under two hours a week. Token ranking performance indicates that cosine similarity comparisons serve as the primary contributors to classifier weighting, with only select diagnostic characteristics features showing significant impact.

Discussion: The development of a method capable of suggesting structure for NPS has the potential to function as an early detection system, supporting their rapid identification and subsequent incorporation into reference libraries. This approach enables timely tracking of shifts within the grey market, while enhancing routine forensic screening through scalable automation suited for laboratory operations. Preliminary validation results are promising for the pipeline's automated ability to prioritize NPS candidates from complex HRMS datasets. Furthermore, leveraging the full power of the LIMS can facilitate the detection of metabolites and underlying relationships efficiently, maximizing the overall utility of the method.

Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

O-125

Risk-Based Evaluation of AI Responses to NPS Synthesis-Related Queries

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Abstract

Introduction: Large language models (LLMs) have significantly advanced chemical research by enabling literature mining, property prediction, and molecular structure generation. However, these systems also raise important dual-use concerns. One key risk is their potential misuse in the clandestine synthesis of new psychoactive substances (NPS), which pose challenges to public health due to rapid structural diversification, toxicity, and evasion of regulatory controls. Synthetic cannabinoid receptor agonists (SCRAs) illustrate this issue, as they often originate from legitimate pharmaceutical research but are redirected for recreational use. The increasing ability to bypass safeguards through jailbreaking techniques highlights vulnerabilities in these systems. In this context, the World Health Organization (WHO) has emphasized the need for ethical AI governance grounded in safety, transparency, accountability, and human oversight.

Objectives: To evaluate the vulnerabilities of LLM-based chatbots in generating information that may facilitate the illicit synthesis of NPS, with a focus on SCRAs, and to identify risk patterns across different models.

Methods: Three independent evaluators with expertise in forensic toxicology and NPS analyzed 60 sanitized prompts designed to preserve scientific context while removing actionable details. Prompts were submitted via API (without account history) to four LLMs: Perplexity, DeepSeek, GPT, and Gemini. Queries were categorized as neutral, ambiguous (dual-use), dangerous, and adversarial (designed to bypass safeguards), and also included tests for hallucination and response reliability. Outputs were assessed using a severity-based framework to distinguish low-risk descriptive content from responses with higher misuse potential.

Results: The models exhibited distinct approaches to balancing safety and engagement. While safeguards generally prevented the disclosure of explicit instructions, procedurally informative content emerged under sanitized or adversarial conditions, potentially lowering barriers to misuse. Severity-based classification proved essential to differentiate these risk levels. Gemini consistently adopted a conservative approach, issuing categorical refusals. Perplexity prioritized conversational continuity, generating low-risk but potentially sensitive outputs. DeepSeek showed variable behavior, including instances of higher-severity structured content. GPT balanced refusal with academic redirection using risk-aware language. A representative case demonstrated that identical prompts can yield markedly different responses across models, ranging from complete refusal to partially informative outputs, including in requests involving procedural descriptions or acquisition-related information.

Discussion: These findings indicate that variability across LLMs can lead to inconsistent risk exposure, raising concerns for public health. This issue is particularly relevant in the absence of harmonized regulatory frameworks. In Brazil, the lack of specific regulation for AI in sensitive domains limits the capacity to address these risks, although ongoing legislative efforts may contribute to mitigation through a risk-based approach, consistent with international trends. Additionally, the absence of clear reporting channels for problematic content and the lack of dedicated oversight bodies may further amplify potential risks. Together, these results highlight the need for stronger governance, continuous stress-testing under adversarial conditions, and interdisciplinary collaboration to ensure the safe and responsible use of LLMs in sensitive areas such as chemistry.

Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

O-126

Development and Evaluation of a Custom Made HRMS Library for Screening Novel Psychoactive Substances in Blood

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Abstract

Introduction: Novel psychoactive substances (NPS) pose a challenge to forensic toxicology laboratories due to their rapid proliferation, structural diversity, and the difficulty commercial databases face in keeping pace. High-resolution mass spectrometry (HRMS) has emerged as a powerful tool enabling compound identification based on accurate mass, isotope pattern, and fragmentation pattern. Additionally, the availability of shared spectral databases further enhances the potential for rapid NPS detection across laboratories.

Objectives: The primary aim of this study was to develop and evaluate an in-house HRMS library for NPS screening in blood. A secondary aim was to contribute our findings to the collaborative highresNPS database.

Methods: A total of 81 compounds spanning seven NPS classes were investigated: synthetic cathinones (n=12), NBOMes (n=8), fentanyls (n=10), semi-synthetic cannabinoids (n=14), synthetic cannabinoids (n=17), nitazenes (n=15), and LSD derivatives (n=6). Library entries were created by injecting reference standards, followed by manual evaluation and curation of fragment ions. Method performance was subsequently evaluated by spiking compounds into blank blood at three concentration levels, with the specific range (0.1–100 ng/mL) selected per compound class based on concentrations reported in literature. Samples were processed by protein precipitation with acetonitrile and analyzed on an Acquity I-Class UPLC coupled to a Xevo G3 QTOF-MS (Waters) operating in data-independent acquisition (DIA; MSe) mode. Identification criteria included retention time deviation (<0.5 min), mass accuracy (<5 ppm), signal intensity (>3,000 counts), isotope pattern match (m/z RMS <20 ppm; intensity RMS <20%), and detection of ≥50% of expected fragment ions.

Results: Fentanyls were reliably identified across all tested concentrations, with three shared fragment ions at m/z 105, 134, and 188. Nitazenes were generally well-identified, with a shared fragment at 107, though sensitivity at 0.1 ng/mL was not consistent across all analytes. NBOMes were readily separated based on retention time and mass, but identification at 0.1 ng/mL, a toxicologically relevant concentration, was not always achieved. Several isomers among the synthetic cathinones (e.g., 2-, 3-, and 4-MMC, and α-PHP/α-PHiP) could not be distinguished by retention time nor by fragmentation. Semi-synthetic cannabinoids showed poor response when spiked in blood, with the exception of 9-α-hydroxy-HHC.

For synthetic cannabinoids, replacing the wash step with an extended gradient improved isotope pattern intensity match scores to acceptable levels, though detection below 10 ng/mL remained challenging. Preliminary analysis of LSD derivative standards suggests high sensitivity, with one shared fragment ion identified at m/z 208.

Advantage of the in-house library has already been demonstrated in a real casework: N-ethylpentedrone was identified in two separate drug seizures, including white crystals recovered from a glove and white crystals from a dealer/user seizure.

Discussion: An in-house HRMS library was expanded with 63 NPS entries across seven drug classes. Manual fragment curation ensures high-quality library entries, while confirming recovery after spiking in blood enables evaluation of method performance at relevant concentrations. The current method is insufficient for reliable semi-synthetic cannabinoid detection in blood. Two unique entries were contributed to the highresNPS database: 9-α-hydroxy-HHC and 25H-NBF. Future efforts will aim to extend the library to additional NPS classes such as 2C-B derivatives and designer benzodiazepines.

Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

O-127

Lab-Quality Portable Microfluidic Platform for Objective Semi-Quantitative On-Site Drug Screening in Urine

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Abstract

Introduction: Reliable preliminary drug screening is crucial in forensic, clinical, and workplace settings. However, conventional lateral flow assays (LFAs) often yield analytical errors, such as false positives or negatives, due to low sensitivity and subjective interpretation. While laboratory systems offer high precision, their size and complexity prevent on-site application. Thus, the growing demand for rapid on-site drug testing urgently requires portable, laboratory-grade technology.

Objectives: To address these gaps, this study developed and validated a lab-quality, on-site drug testing device utilizing a fluorescence-based microfluidic assay. The system's performance was rigorously evaluated using 141 authentic urine specimens obtained from the National Forensic Service (NFS) in Korea and Lab HORVATH in Paraguay. This research offers a practical hybrid solution for methamphetamine (MET), benzoylecgonine (BE), 11-nor-9-carboxy-tetrahydrocannabinol (THC-COOH), and benzodiazepines (BZOs), facilitating rapid decision-making in diverse forensic environments.

Methods: A capillary-driven microfluidic chip was engineered for autonomous fluid transport, incorporating a two-channel architecture with drug-specific antibodies and fluorescent beads for simultaneous multi-analyte detection. A compact portable reader was developed to provide objective measurements by quantifying relative fluorescence units (RFU). For analytical validation, the assay was evaluated against standard drug concentrations to determine the limit of blank (LoB), limit of detection (LoD), and cross-reactivity, ensuring specific detection at target cutoff levels. Clinical performance was then assessed using 141 authentic urine specimens. The results were cross-validated against standard reference methods, including the COBAS c311 analyzer and rapid test kits, with complex cases, such as polydrug use, subsequently confirmed via Gas Chromatography-Mass Spectrometry (GC-MS).

Results: The optimized microfluidic assay achieved simultaneous detection of the four target drugs within 5 minutes. Instrumental validation confirmed that the LoD for all analytes were established well below their respective standard cutoff concentrations (MET: 1000 ng/mL, BE: 300 ng/mL, THC-COOH: 25 ng/mL, BZOs: 300 ng/mL), with no cross-reactivity observed among the drugs. When applied to 141 authentic urine specimens, the device provided objective, semi-quantitative data via the cutoff index (COI). For MET, BE, and THC-COOH, the assay yielded zero false positives and zero false negatives, showing perfect correlation with standard reference methods. Furthermore, the system successfully identified 36 polydrug use cases without interference. Although BZOs exhibited a lower detection rate (46%) due to extensive metabolic diversity, the assay consistently maintained a zero false-positive rate across all samples.

Discussion: This microfluidic platform effectively bridges the gap between point-of-care testing and laboratory-based methods. The assay demonstrated reliable simultaneous detection of all four target drugs within 5 minutes. While MET, BE, and THC-COOH achieved perfect accuracy, BZOs also consistently maintained a zero false-positive rate, underscoring the system's overall high specificity. By providing objective, semi-quantitative data through the COI, this device overcomes the subjective visual interpretation of traditional lateral flow assays. Ultimately, this easy-to-use, portable, and cost-effective solution offers a lab-grade screening tool for on-site applications. Future research will focus on expanding the drug panel and improving detection limits for trace metabolites.

Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

O-128

Identification of Δ9-THCB, Δ9-THCH, and Δ9-THCP Phase I Biomarkers and Minor Metabolites with Human Liver Microsomes

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Abstract

Introduction: Following the 2018 Agricultural Improvement Act, there has been an increase in the availability of novel Δ9-tetrahydrocannabinol (THC) analogs, many of whose urinary biomarkers remain unidentified. Δ9-tetrahydrocannabutol (Δ9-THCB), Δ9-tetrahydrocannabihexol (THCH), and Δ9-tetrahydrocannabiphorol (THCP) are structurally similar to Δ9-THC and differ only in alkyl side chain length (THCB = 4; THC = 5; THCH = 6; THCP = 7). Characterizing the metabolic profiles and identifying biomarkers for these compounds is essential for developing analytical methods to accurately assess exposures and inform regulatory decision-making.

Objectives: To presumptively identify Δ9-THCB, Δ9-THCH, and Δ9-THCP phase I biomarkers and minor metabolites *in vitro* by fragmentation pattern interpretation using multiple reaction monitoring (MRM) and precursor ion (PI) scanning.

Methods: In brief, metabolism controls (0 ng/mL), samples (800 ng/mL), and a positive control (Δ9-THC; 800 ng/mL) were incubated with human liver microsomes in phosphate buffer (pH 7.4) containing nicotinamide adenine dinucleotide phosphate and magnesium chloride at 37 °C. Reactions were quenched with ice-cold acetonitrile at multiple time points (0–240 minutes) to characterize metabolic profiles. Extracts were evaporated under nitrogen and reconstituted in methanol with internal standard prior to analysis. Liquid chromatography–tandem mass spectrometry (LC–MS/MS) was performed using a SCIEX Triple Quad 6500+ with chromatographic separation on a Zorbax Eclipse XDB-C18 column under isocratic conditions (water:methanol, 10:90 v/v, 0.1 mM ammonium formate, 1.0 mL/min). Data were acquired in positive ion mode (100–500 m/z) using multiple reaction monitoring (MRM) and precursor ion (PI) scanning. Theoretical MRM transitions and PI product ions were based on structural similarities to Δ9-THC and its primary metabolites, 11-hydroxy-Δ9-THC and 11-nor-Δ9-carboxy-THC, with PI scans conducted over precursor ions of 100–450 Da and collision energies of 15–35 V.

Results: Using MRM, predicted biomarkers 11-hydroxy-Δ9-THC[B,H,P] (11-OH-THC[B,H,P]) and 11-nor-Δ9-carboxy-Δ9-THC (11-COOH-THC[B,H,P]) were identified. However, hydroxylation at the eleventh position decreased with increasing alkyl side chain length, with conversion rates declining from Δ9-THC (83%) to Δ9-THCB (45%), Δ9-THCH (40%), and Δ9-THCP (29%). Precursor ion (PI) scanning identified additional potential metabolites structurally similar to minor Δ9-THC metabolites. For Δ9-THCB, two metabolites were observed: A4 (hydroxyl/epoxy, 317 m/z) and B4 (dihydroxylation, 333 m/z, with suggested dual carboxylation, 345 m/z), supported by intermediate ions (347 m/z). For Δ9-THCH, metabolites A6 (hydroxylation, 345 m/z) and B6 (dihydroxylation followed by ketone/carboxylic acid formation, 373 m/z) were presumptively identified, with supporting intermediates. Δ9-THCP produced a metabolite involving hydroxylation (359 m/z) and subsequent carboxylation (373 m/z). Further structural confirmation of these metabolites is required.

Discussion: The availability of novel Δ9-THC analogs has created a need to establish metabolic profiles to accurately assess exposures and inform regulatory decision-making. The presented methods provide a practical approach for identifying metabolites and biomarkers. Although in vivo studies and reference materials are needed to fully evaluate metabolite formation, this in vitro analysis is an important first step toward developing reliable forensic detection methods that could be applicable to authentic specimens.

Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

Conflict of Interest

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O-129

Evaluation of Acetone as a Biomarker for Alcohol Involvement in Drug-Facilitated Sexual Assault Cases

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Abstract

Introduction: Alcohol is the most commonly encountered substance in drug-facilitated sexual assault (DFSA) investigations. However, delayed reporting and the relatively rapid elimination of ethanol often results in negative alcohol findings in whole blood and urine samples collected during forensic examination. This presents a significant challenge for toxicological interpretation and has prompted interest in alternative biomarkers that may provide additional evidence of trauma.

Objectives: The aim of this study was to develop and validate an analytical method for the quantification of acetone in biological samples and to evaluate its potential as a supportive biomarker of alcohol involvement in DFSA investigations 8 - 72 hours post incident.

Methods: A headspace gas chromatography–flame ionisation detection (HS-GC-FID) method was developed for the simultaneous determination of acetone and ethanol in whole blood and urine. Method validation was performed in accordance with ISO/IEC 17025:2017 requirements. The acetone calibration range (5 – 300 mg/L) was prepared in an aqueous solution containing sodium metabisulphite, 1-propanol, and potassium carbonate. Matrix matched QCs were tested during validation, and validation parameters included: linearity, accuracy, precision, measurement uncertainty, selectivity, limit of detection (LOD), limit of quantitation (LOQ), matrix effects, and evaluation of salting-out effects.

Results: The method demonstrated linearity across a concentration range of 5 – 300 mg/L of acetone with correlation coefficients of > 0.990. The limit of detection for acetone was 2.5 mg/L. Accuracy was less than 20%, with intermediate precision values of 3 – 8%. The expanded measurement uncertainty at 95% confidence was less than 20%.

To assess the forensic relevance of acetone, over 100 DFSA case samples were evaluated. Acetone was detected in 26% of cases where ethanol was present and in 33% of cases where ethanol was not detected at the time of analysis. Acetone concentrations ranged from 5 – 30 mg/L in blood and 5 – 240 mg/L in urine. Elevated acetone concentrations were observed in cases involving alcohol consumption with sample collection delays of > 10 hours.

Discussion: These findings demonstrate that acetone may provide supportive evidence of trauma involvement in DFSA investigations as an adjunct to alcohol analysis. The development of validated analytical methods and case-based interpretation criteria may enhance toxicological interpretation and provide additional evidential support in DFSA casework.

Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

O-130

Analytical Confirmations of Emergency-Department Intoxications from April 2022 – March 2026 in Victoria, Australia

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Abstract

Introduction: In the state of Victoria, Australia, selected emergency department (ED) cases of suspected or reported illicit drug exposure that undergo comprehensive toxicological analysis are de-identified and included within the Emerging Drugs Network of Australia-Victoria (EDNAV) registry. Fourteen metropolitan and four regional EDs contribute data to the registry. Each week up to 22 cases are purposively analysed, collating clinical and toxicological data and enabling emerging drug use in the community to be linked to clinical presentations and health outcomes. This project functions as an early warning system, with drug alerts disseminated to health and peer support workers.

Objectives: To determine the frequency of drug detections, and changes in drug detection frequencies over the prior four-year period (April 2022-March 2026) of EDNAV casework.

Methods: EDNAV casework is screened using targeted liquid chromatography-triple-quadrupole-mass spectrometry (LC-MS/MS) methods for 327 common drugs and poisons and 324+ novel psychoactive substances (NPS), and for HighResNPS.com database targets using liquid chromatography-time-of-flight-mass spectrometry (LC-QTOF-MS). All cases undergo LC-MS/MS quantitation of gamma-hydroxybutyrate (GHB). Analytical data was examined for 4,439 cases over the study period. Alcohols are not comprehensively analysed and so are not included in this analysis.

Results: 238 different analytes were detected (221 parent/metabolite pairs) in the 4,439 cases. There were 52 analytes which were detected once and all in different cases, usually identified as an NPS. The most common drugs detected were methylamphetamine (MA) (69% of total cases), diazepam (48%), GHB (38%), delta-9-tetrahydrocannabinol (21%) and ketamine (21%). GHB median concentration was 130 mg/L, with ~5% of detections exceeding 370 mg/L and three > 1000 mg/L. The most common co-detections with MA were diazepam (53% of MA-positive cases) and GHB (52% of MA-positive cases). NPS were detected in 22% of cases, with the most frequent classes detected: novel benzodiazepines (n=1259 detections, predominantly bromazolam: n=502, the 10th most common drug detected in the cohort at 11% of total cases), cathinones (n=192, predominantly pentylone or dimethylpentylone (n=52) from Q3 2022-Q4 2023 or methylone (n=48)), and phencyclidine-type substances (n=91, predominantly 2'-fluoro-2-oxo-PCE (n=41) from Q4 2023-Q4 2025). MA was detected in most GHB-positive cases (94%) and bromazolam-positive cases (73%). The most frequently co-detected drugs in pentylone and dimethylpentylone-positive cases were MDMA (54%), MA (46%) and diazepam (46%), and in 2'-fluoro-2-oxo-PCE cases the most frequently co-detected drugs were ketamine (61%) and cocaine (46%). 71 cases were found to be drug free.

Discussion: Due to project constraints, cases are included only where there is a particular suspicion of emerging drug use or heightened public health risk. Even with this bias, traditional drugs of misuse dominated the cohort compared to NPS, particularly MA and GHB. Notably, no fentanyl analogues were detected, contrasting with global toxicology findings. The identification of illicit drugs taken in individuals who require emergency-department management provides knowledge of illicit drugs in the community that may cause harm, patterns of evolving drug use, and informs harm reduction policy. MA, GHB, and novel benzodiazepines were consistent drugs of concern over the past four years in Victoria, with an apparent transient emergence of cathinone and phencyclidine-type substances.

Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

O-131

Can a Single Dose Leave a Trace? Interpretation of Sedating Antihistamines in Hair After Single-Dose Exposure and Application to Drug-Facilitated Crime Cases

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Abstract

Introduction: Drug-facilitated crimes (DFCs) remain a challenge in forensic toxicology. First-generation sedative antihistamines (e.g., hydroxyzine, diphenydramine, doxylamine) are frequently implicated due to their central nervous system depressant effects. Their short detection window in conventional matrices complicates delayed sampling, making hair analysis the only retrospective evidence. However, interpreting a single exposure is hindered by a lack of reference data on drug incorporation, complicating the distinction between one-time administration and chronic use.

Objectives: This study aimed to evaluate the detectability of selected sedating antihistamines (hydroxyzine, diphenhydramine and doxylamine) in hair after single exposure and to assess the relevance of these data in interpreting suspected DFC cases.

Methods: Hair samples were analyzed using an LC-MS/MS method (Altis, Thermo Fisher Scientific®). A controlled administration study was conducted for hydroxyzine and diphenhydramine: three volunteers (two females with dark hair and one male with blond hair) received a single oral dose of 25 mg hydroxyzine and 90 mg diphenhydramine. Despite the limited sample size, these findings represent the first controlled administration data in hair and provide an important reference for the interpretation of DFC investigations. Hair was collected one month post-administration. Samples underwent decontamination, overnight incubation in a basic buffer (pH 9.5), and extraction prior to analysis. For doxylamine, interpretation relied on previously published data (1). The method was subsequently applied to real DFC cases analyzed between July 2025 and March 2026.

Results: In the controlled study, hydroxyzine concentrations after a single dose were 59–63 pg/mg in dark hair and 15 pg/mg in blond hair. Diphenhydramine concentrations ranged from 311 to 400 pg/mg in dark hair and 31 pg/mg in blond hair. In real cases, hydroxyzine was detected in 7 cases (2.7–1141 pg/mg), diphenhydramine in 10 cases (0.7–266 pg/mg), and doxylamine in 14 cases (1–950 pg/mg). Literature data indicate doxylamine concentrations of 18–52 pg/mg following a single dose.

Discussion: The study provides reference values that improve interpretation of hair analysis in DFC investigations. Nevertheless, interpretation should also take into account segmental concentration patterns and other case-specific variables. For hydroxyzine, three cases (2.7–19 pg/mg) were consistent with single exposure, while higher concentrations (128–1141 pg/mg) suggested repeated use. For diphenhydramine, most case concentrations were below controlled single-dose levels; notably, one case (266 pg/mg) remained compatible with single exposure and should not be considered unusually high. For doxylamine, most cases aligned with single use, whereas higher concentrations (338 and 950 pg/mg) indicated chronic intake. Hair pigmentation significantly influenced drug incorporation, with higher levels observed in dark hair due to

melanin affinity, increasing the risk of false negatives in individuals with light hair. Conversely, relatively high concentrations after a single dose, particularly for diphenhydramine, may be misinterpreted as repeated use in the absence of appropriate reference data. Combining controlled administration studies with real casework enhances the interpretation of hair findings in DFC investigations. These data provide practical thresholds to differentiate single from chronic exposure, strengthening the evidential value of hair analysis.

1: Kintz P, Villain M, Cirimele V. Hair analysis for doxylamine. Forensic Toxicol. 2012;30(1):1–5

Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

O-132

Oral Fluid Test Results After Acute and Chronic Dosing of a Topical Hemp Product in Uncontrolled and Controlled Settings

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Abstract

Introduction: The 2018 Farm Bill legalized retail hemp products, which has complicated the landscape of workplace drug testing programs. These programs commonly analyze various biological matrices for delta-9-tetrahydrocannabinol (THC) and its metabolites to infer recent cannabis use. Many hemp products contain THC, but this is often not disclosed to the consumer on product labeling. Moreover, the topical application of THC containing hemp products (e.g., lotion) could result in contamination of biospecimens if hands are not properly sanitized after application. Lymphatic deposition of cannabinoids (e.g., THC) may also occur via transdermal absorption from upper arm areas.

Objectives: A two-study series aims to delineate the impact of hemp-derived high cannabidiol (CBD)/low THC topical products on the drug testing of oral fluid (OF) samples. Study 1 characterized the pharmacokinetic and pharmacodynamic profiles of hemp-derived topical cannabinoid products using blood, urine, and OF samples. Based on Study 1 hemp lotion results, Study 2 tests an incidental OF contamination hypothesis.

Methods: Study 1 used a double-blind, between-subjects design where participants were randomized to use one of five topical products (lotion, cream, patch, balm, or gel), which contained either high CBD/low THC or placebo. Pharmacokinetics (blood, urine, OF) and pharmacodynamics (subjective, cognitive, and physiological effects) were assessed in an acute laboratory session; during a 10-day outpatient dosing period with twice daily application; and after a 7-day washout.

Study 2 is a 7-day residential study using the same high CBD/low THC lotion from Study 1. The lotion is applied to the participants' upper arms four times daily in a sanitized and controlled procedure that eliminates the potential for specimen contamination. OF is collected before and after application (six samples/day); blood and urine are collected once/day. On discharge day, participants provide an intentionally contaminated OF sample. Participants return after a 7-day washout to provide 4 additional OF specimens, one "clean" and three more intentionally contaminated samples over a two-hour course.

Results: In Study 1 (N=46), eight participants were randomized to the active lotion condition. Seven of the eight participants handled and provided OF samples positive for THC (≥ 2 ng/mL), but no THC or metabolites were detected in blood or urine samples. THC concentrations in OF ranged from 2 to 12 ng/mL and mainly occurred on Day 7 and/or Day 10 study visits (no positive tests observed at washout). No discernible pharmacodynamic

effects were found across active conditions. Study 2 is currently ongoing with a target sample of 6 participants to complete the study. Biospecimen analyses are pending; results are expected no later than August 2026.

Discussion: Study 1 found positive THC results after repeated exposure to a hemp-derived lotion, but only in OF. Study 2 seeks to contextualize these previous findings by testing an incidental oral contamination hypothesis through dosing in a controlled, sanitized environment. These data will enhance understanding as to whether federally legal hemp-derived topical products can impact drug testing for cannabis and will determine whether positive results are likely due to biospecimen contamination as opposed to other theories, such as lymphatic deposition via transdermal absorption.

Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

O-133

Establishment of a National Drug Checking Laboratory: Support Toxicological Surveillance in Scotland

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Abstract

Introduction: Scotland continues to experience disproportionately high levels of drug-related harm and mortality. It has consistently reported the highest drug-related death rate in Europe and is second only to the United States globally in terms of drug mortality rates. These harms are driven by an increasingly complex and volatile illicit drug market characterised by poly-drug mixtures, fluctuating potency, and the emergence of new psychoactive substances. In response, the Scottish Government has supported a multi-city, multi-agency, drug-checking pilot aimed at providing individuals with actionable information about substances while generating intelligence to inform public health and policy responses. Central to this initiative is the establishment of a National Testing and Research Laboratory (NTRL) at the Leverhulme Research Centre for Forensic Science (LRCFS), University of Dundee.

Objectives: To (i) outline the workflow linking point-of-care testing sites to the central laboratory; (ii) describe the analytical strategies employed for rapid and confirmatory testing; and (iii) explore how the system can support both individual harm reduction and wider surveillance of the illicit drug supply.

Methods: The pilot is being implemented across multiple community-based sites where individuals can voluntarily submit small quantities of substances for analysis. Upon receipt, samples are divided into two portions. One portion undergoes rapid, on-site screening using portable analytical technologies to provide immediate, indicative results, which are communicated to the individual as part of a harm reduction consultation. The second portion is securely packaged and transported under controlled conditions to the NTRL. At the laboratory, samples undergo confirmatory analysis using gas chromatography mass spectrometry and high-performance liquid chromatography coupled with a diode array detector. Data generated from both on-site and laboratory analyses are recorded and integrated to support emerging monitoring and trend analysis.

Results: Lessons learned throughout the development and early implementation of the pilot will be communicated, with a focus on the practical considerations of establishing a coordinated drug-checking system linking point-of-care services with a centralised laboratory. This will include reflections on workflow design, sample handling, transport logistics, and data integration processes. Initial data collected will be presented to illustrate early system outputs.

Discussion: The development of the NTRL represents an important step in strengthening Scotland's harm reduction infrastructure by linking community-based services with forensic analysis. This approach enables timely, person-centred interventions while also supporting the generation of high-quality data about the illicit drug market in Scotland. For forensic toxicology services, this model offers a significant advantage by providing a robust and current evidence base to guide decisions on which substances to target, thereby improving the relevance, sensitivity, and efficiency of toxicological analyses.

Disclosure

Yes, I, or a member of my immediate family, has a financial interest to disclose.

Conflict of Interest

This work is funded by the Scottish Government

O-134

Scopolamine Detection Following N-Butylscopolamine Administration: Toxicological Considerations in DFC and DFSA Cases

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Abstract

Introduction: Scopolamine is a tropane alkaloid present in many plants of the Solanaceae family. It possesses antimuscarinic activity at the central nervous system level as well as throughout the body. In the forensic lab context scopolamine is often investigated when a drug facilitated crime (DFC) scenario is suspected, including drug facilitated sex assault (DFSA), as it may cause confusion, agitation, delirium, anterograde amnesia and submissive behavior. However, based on our experience and on the consulted literature, actual detection of scopolamine related to these cases is anecdotal. On the other side, N-butylscopolamine is a frequently used antispasmodic drug generally used for the symptomatic treatment of abdominal cramping and pain. Despite their structural similarity, they differ markedly in physicochemical properties and are not metabolically related to our knowledge.

Objectives: The finding of scopolamine in a DFSA oriented case in our lab and the subsequent detection of N-butylscopolamine raised a red flag about the correct interpretation of the results in these cases. The aim of this work was to determine the most adequate lab protocol specifically for DFC/DFSA oriented cases where scopolamine is found.

Methods: Blood and urine samples of a 33-year-old female with a medical history of breast cancer and possible victim of a DFSA in a restaurant were received in our lab. The time interval between the alleged events and the sample collection was approximately 18 hours.

Samples were initially processed following our standard lab procedure for general toxicological screening, including enzyme immunoassay (CEDIA), ethanol determination and a solid phase extraction (SPE) before analysis with a UHPLC-HRMS-Q-Orbitrap system as well as HPLC-DAD and GC-MS.

After the initial findings, the remaining urine sample was diluted and directly analysed with a LC/MS QTOF system. A blood sample submitted to ultrafiltration was also additionally investigated by UHPLC-HRMS-Q-Orbitrap.

Results: The immunoassay screening was negative. No ethanol was detected in blood and 0.14 g/L ethanol was detected in urine. Traces of scopolamine as well as metoclopramide and its metabolites were detected in the SPE extracted urine sample. When the remaining urine sample was pretreated using a dilution protocol, N-butylscopolamine was additionally detected. The proportion of scopolamine to N-butylscopolamine based on the areas was approximately 1:400.

Traces of metoclopramide were detected in the SPE extracted blood sample, but neither scopolamine nor N-butylscopolamine were found. However, after ultrafiltration of the blood sample, traces of N-butylscopolamine were detected.

Discussion: Due to its chemical nature N-Butylscopolamine might escape protocols where only SPE is routinely performed. In cases where scopolamine is detected, especially if DFC is suspected, the presence of N-Butylscopolamine should be specifically investigated as the finding of the former might be associated with the presence of the latter. Thus, a protocol including dilution or ultrafiltration pretreatments combined with SPE may be of choice to avoid possible misinterpretation of the results. Our lab is currently investigating the origin of scopolamine traces associated with N-butylscopolamine.

Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

O-135

Integrating Self-Reported and Biological Measures to Assess Psychoactive Substance Use Among Medical Students in Italy: The NO PANIC Study

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Abstract

Introduction: Psychoactive substance use and neuroenhancer consumption among university students represent a growing public health concern, particularly in high-pressure academic environments such as medical schools. However, existing evidence largely relies on either self-reported data or biological analyses, each with inherent limitations.

Objectives: This study aims to estimate the prevalence and patterns of psychoactive substance use among medical students during examination periods and to evaluate the agreement between self-reported and biologically detected substance use.

Methods: A multicenter cross-sectional study was conducted among medical students from two Italian universities (University of Pavia and University of Bologna) during examination sessions. Data collection included a structured self-administered questionnaire assessing substance use patterns, motivations, and psychological factors. Dried blood spots (DBS) and hair samples were collected to evaluate recent and long-term substance exposure, respectively. Toxicological analyses were performed using validated LC–MS/MS methods targeting a broad panel of psychoactive substances, including recreational drugs, prescription medications, neuroenhancers, and new psychoactive substances. Integrated prevalence was calculated by combining self-reported and biological data.

Results: Preliminary data from one study site (Pavia) included 180 participants. No neuroenhancers or new psychoactive substances (NPS) were detected in any biological samples. Overall biological positivity for psychoactive substances was observed in 23 participants (12.8%). Self-reported substance use showed that 14 participants reported weekly or near-daily consumption and a further 12 reported monthly use, whereas 53 reported use only once or twice during the reference period. The prevalence of biological positivity was therefore broadly consistent with self-reported regular substance use. The most frequently detected substances were cannabinoids (7; 3.9%), benzodiazepines (7; 3.9%), cocaine (4; 2.2%), and antidepressants (4; 2.2%).

Discussion: Comparison with self-reported data revealed substance-specific patterns of agreement and discrepancy. Cocaine use was not self-reported by any participant, yet it was detected in 4 cases through biological analysis, indicating complete underreporting. In contrast, cannabis use was self-reported by 50 participants (27.8%), predominantly as occasional consumption. Only 3 participants reported near-daily use and 9 reported monthly use, whereas 38 reported use only once or twice during the reference period. THC was detected in hair samples from 7 participants (3.9%), suggesting that biological testing more closely reflected regular rather than occasional cannabis use. Antidepressants showed partial agreement between self-reported and toxicological findings. Six participants

reported daily or near-daily antidepressant use, whereas antidepressants were analytically confirmed in only two cases.

Regarding legal substances, caffeine and nicotine were widely detected and consistently self-reported across participants. Daily or near-daily consumption was reported by 115 participants (63.9%) for caffeine and 36 participants (20.0%) for nicotine. In contrast, daily alcohol consumption was reported by only one participant. Consistently, EtG concentrations in hair were below 10 pg/mg in all participants.

Conclusion: This study demonstrates the value of integrating self-reported and biological measures for assessing psychoactive substance use among medical students. The combined approach identified discrepancies between reported and analytically confirmed use, particularly for cocaine and cannabis, highlighting the limitations of relying on a single source of information. These findings may support more accurate epidemiological assessments and inform targeted prevention strategies in academic settings.

Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

O-136

Neurochemical Effects of Chronic Ketamine Exposure: Insights from Mass Spectrometry Imaging and Metabolomics

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Abstract

Introduction: Ketamine, an N-methyl-D-aspartate (NMDA) receptor antagonist, exhibits both therapeutic potential and abuse liability. However, the spatial distribution of ketamine in brain regions and the pattern of metabolite changes induced in the corresponding brain regions are not clear. This study combined ethological tests with mass spectrometry imaging (MSI) and metabolomics to investigate ketamine's spatial distribution and metabolic impact in key brain regions in a mouse model.

Objective: To investigate the spatial distribution of ketamine in the brain and its region-specific metabolic alterations underlying ketamine-induced anxiety in a mouse model using MSI and metabolomics methods.

Method: C57BL/6 mice received daily ketamine (30 mg/kg, n=15) or saline (10 mL/kg, n=15) for 15 days. Ethological tests (Elevated Plus Maze, Open Field, New Object Recognition, Y-maze) were applied to assess the behavioral manifestations, as well as evaluate the severity of anxiety and cognitive impairment. Desorption Electrospray Ionization (DESI) MSI was used to map ketamine and γ-aminobutyric acid (GABA) spatial distributions. Data were acquired using a quadrupole time-of-flight (qTOF) mass spectrometer (MS) and imported into High-Definition Imaging (HDI) software for peak extraction and imaging analysis. Based on MSI results, specific brain regions were selected for metabolomics analysis via Ultra-Performance Liquid Chromatography coupled with Quadrupole-Orbitrap MS. The MS data was acquired in data-dependent acquisition (DDA) mode. The databases HMDB, Metlin, KEGG, mzCloud, and a self-constructed standard database were applied for metabolite identification. The differential metabolites were screened and submitted to pathway analysis.

Results: Ketamine-treated mice showed increased anxiety-like behaviors ($p<0.05$), which was evidenced in the Elevated Plus Maze and the Open Field Test. However, no cognitive deficits were observed in New Object Recognition and the Y-maze. MSI revealed that ketamine predominantly accumulated in the cerebral cortex, midbrain, and cerebellum, while GABA showed a distinct redistribution with higher abundance in the thalamus and striatum. Based on these spatial findings, the prefrontal cortex (PFC) and cerebellum were selected as target regions for metabolomics analysis. Compared to saline-treated controls, ketamine treatment resulted in 73 differential metabolites in the PFC (24 upregulated, 49 downregulated) and 134 differential metabolites in the cerebellum (118 upregulated, 16 downregulated). Differential metabolites were primarily involved in the Estrogen signaling pathway, glutamatergic and GABAergic signaling pathways, as well as GABAergic synapse pathways.

Discussion: This study reveals that ketamine-induced anxiety arises from region-specific neurochemical disruptions. MSI showed ketamine accumulation in the cortex and cerebellum, while GABA redistributed to the thalamus and striatum, suggesting that ketamine acts on proximal regions to modulate GABAergic circuits. Metabolomics further showed that in the PFC, ketamine disrupted glutamate/GABA metabolism and reduced N-acetylaspartate (NAA), indicating impaired neuronal integrity and excitatory-inhibitory imbalance, which likely weakens cortical regulation of the amygdala to promote anxiety. In the cerebellum, ketamine altered glutamatergic pathways, potentially affecting cerebellar output circuits that control anxiety. Additionally, enrichment of the Estrogen signaling pathway in male mice suggests that ketamine disrupts steroid homeostasis, probably via ERα and ERβ mechanisms. Together, ketamine induces brain region-specific metabolic reprogramming of glutamate/GABA balance and estrogen signaling, providing a potential mechanistic basis for its anxiety-inducing effects.

Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

Conflict of Interest

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O-137

Evaluation of Sexual Assault Cases in Houston: A Six-Year Update (2019-2024)

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Abstract

Introduction: In 2024, 6.7 million violent crimes of people aged 12+ occurred nationally. A significant increase (32%) in these crimes was noted in 2020 which subsequently stabilized in 2022. Violent crimes include sexual assault (SA), robbery, and assault with/without a deadly weapon. SA cases encompass 8.4% of the national violent crime victimizations.[1]

Less than 50% of violent crimes committed were nationally reported in 2024 based on a self-report survey.[1] Of those cases, less than 24% were SAs. These crimes are often not reported for fear of repercussions, believing the justice system cannot help, and thinking it is a personal issue or too insignificant to report.[1] Furthermore, because of the nature of SA cases, there is often a delay due to the victim being incapacitated by alcohol and/or drugs.

Objectives: The purpose of this study was to evaluate the Houston Forensic Science Center (HFSC) SA drug trends and related demographics from 2019 to 2024.

Methods: Ethanol analysis was performed in blood for SA cases using headspace gas chromatography (GC). Drug screening using immunoassay and GC-mass spectrometry (MS) and confirmation using liquid chromatography-MS/MS were conducted in urine (where available) due to longer drug detection windows and extended delays in collection. Data were compiled from January 2019 to December 2024 and were evaluated for trends including alcohol positivity, high concentration alcohols (>0.200 g/100mL), alcohol and drug co-administration, and drug only positivity. In addition, gender and race/ethnicity demographics were reviewed.

Results: HFSC SA cases encompassed an average of 5% of all case submissions from 2019-2024; 78% of cases were negative. Alcohol was the most detected drug with an average prevalence of 20%, and high concentration alcohols averaged 7%. Coadministration of alcohol and drugs was found in a maximum of 3% of cases in 2019. Drug positive only cases had an average prevalence of 1%. Multiple drug classes were identified with/without alcohol including amphetamines (2.9%), antidepressants (0.4%), antihistamines (0.5%), benzodiazepines (2.1%), cocaine/metabolites (1.1%), opioids (1.6%), and PCP (0.8%). Gender distribution averaged 52% female, 43% unknown, and 5% male. The average race/ethnicity breakdown was 1% Asian, 3% Hispanic, 9% black, 11% white, 36% other, and 41% unknown.

Discussion: From 2019 to 2024, HFSC saw a decrease of 53% of toxicological casework submitted (DWI, SA, and other offenses); however, SA casework slightly increased by 6% from 2019. A total of 1400 SA cases were analyzed from 2019 to 2024 (230 cases/year). Most victims were female and either black or white. However, these demographics could often be limited to maintain anonymity and because of routine clinical collection.

Alcohol was the most encountered drug because it is often voluntarily consumed by victims. Alcohol positivity increased by 3% from previous years (2014-2020). Amphetamines and

benzodiazepines were the most common compounds detected with alcohol which is a significant change from marijuana metabolite (2014-2020). Examining and discussing SA drug trends and demographic data promote public awareness and aid in investigations.

References

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Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

O-138

Drug Residue Analysis of Discarded Paraphernalia: 12-Month Spatio-Temporal Patterns From South Australia

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Abstract

Introduction: Residue analysis of discarded paraphernalia from people who inject drugs (PWID) provides a novel and unobtrusive approach to investigate drug use behaviours. This method enables anonymous sampling without influencing behaviour or relying on voluntary drug checking. The study followed on form a pilot that was conducted in late 2024. Paraphernalia were collected from six sites across South Australia over a 12-month period to characterise spatio-temporal patterns in substance use. Analysis targeted both conventional drugs and novel psychoactive substances (NPS), generating insights into evolving drug use trends and the emergence of new substances.

Methods: Sharps disposal bins located at local clinics operating needle exchange programs were collected from six metropolitan sites across South Australia by a service provider and returned to a central location over a 12-month period in 2025. The program met all ethics requirements and was conducted with approval from the Community Advisory Committee of the Drug and Alcohol Services of South Australia (DASSA). A total of 900 items were retrieved, including syringes, bags, drug storage vials/containers, and filters. Samples were rinsed with methanol and analysed by LC–MS/MS (SCIEX 6500+ QTRAP), with detections confirmed against authentic reference materials.

Objectives:

- To characterise injecting drug use patterns in South Australia, including the detection of high-potency novel psychoactive substances (NPS).
- To investigate demographic differences in drug use and changes over time
- To monitor the presence of nitazenes and other high-risk NPS in drug paraphernalia as indicators of emerging public health threats.
- To evaluate the feasibility and effectiveness of drug residue analysis as a non-invasive method for near real-time surveillance of the illicit drug market.

Results: Of the 900 samples analysed, 734 syringes (81%), 145 plastic bags (16%) and 21 other items (3%) including filters, straws, and other drug packaging were analysed. In total 17 different drugs were detected over the collection period. A high proportion of samples contained methamphetamine (65%) and/or heroin (58%), while other commonly detected substances included cocaine (35%) and methadone (11%). Most of the items analysed contained two or more substances (70%). Across all samples, 15 different nitazenes were detected representing an overall detection frequency of 10%. Temporal trends demonstrated a general upward trend in detections of methamphetamine, heroin and cocaine over the sampling period.

Discussion: A high number of items containing multiple drugs likely reflects polysubstance use or the reuse of needles and syringes, though adulteration may also contribute. 10% of all samples contained nitazenes. Of note 50% of nitazene positive samples contained heroin as the major constituent, whilst 48% contained methamphetamine. This study provides analytical confirmation of the substances being injected, offering insights that cannot be captured through existing methods such as self-reported use or population-level monitoring. Importantly. It also provides valuable information on temporal trends and spatial patterns across jurisdictions, helping to identify emerging substances and shifts in use over time and between locations. Residue analysis of drug paraphernalia is an objective approach that complements current methods for identifying drug trends, strengthening harm reduction efforts and informing targeted public health responses to illicit drug use.

Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

O-139

Rapid LC-HRMS-Based Nontargeted Analysis of Emerging Nitazene and Orphine Opioids in Human Urine

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Abstract

Introduction: The synthetic opioid landscape is evolving at a pace that is increasingly misaligned with the capabilities of conventional forensic and clinical toxicology workflows. Highly potent new psychoactive substances (NPS), particularly nitazene-class benzimidazole opioids, have rapidly emerged in illicit drug markets and are now among the most potent opioid substances associated with fatal overdoses. In parallel, structurally related orphine-type opioids (benzimidazol-2-one analogs) are appearing following regulatory scheduling, often without reference standards or established toxicological profiles. These compounds are frequently encountered in adulterated and substituted drug supplies, where their presence is unexpected and clinically consequential. A critical analytical challenge is their extensive structural similarity and isomerism, which significantly reduces the effectiveness of traditional targeted screening strategies. As a result, there is a growing demand for analytical workflows that can simultaneously deliver high-throughput performance, structural resolution, and adaptability to unknown or emerging analogs within complex biological matrices.

Objectives: This study aims to develop a rapid, high-throughput, nontargeted analytical workflow for the detection and structural characterization of nitazene and orphine-type opioids in human urine. Using LC-HRMS with data-dependent acquisition, the goal is to enable comprehensive screening and differentiating structurally similar compounds. Particular emphasis is placed on resolving nitazene isomers and establishing characteristic fragmentation patterns to support confident identification in complex biological matrices.

Methods: A panel of 23 synthetic opioids (20 nitazenes and 3 orphine-type compounds) was evaluated alongside an internal standard. Drug-free human urine was fortified to generate an eight-point calibration curve (0.5-100 ng/mL) and prepared using a dilute-and-shoot approach with four-fold dilution. Analysis was performed on an Agilent 1290 Infinity LC system coupled to a 6546 QTOF mass spectrometer with dual Jetstream electrospray ionization in positive mode. Chromatographic separation was achieved using an optimized gradient with a 4.5-minute runtime. Data were acquired using a non-targeted, data-dependent acquisition strategy. Structural elucidation was supported by MS/MS fragmentation via collision-induced dissociation at multiple energies (10, 20, and 30 eV). Data processing was conducted using MassHunter Qualitative Analysis and Quant-My-Way software.

Results: The method demonstrated strong analytical performance, combining rapid separation with robust structural characterization. Nineteen of twenty nitazene isomers were resolved within the 4.5-minute runtime. Mass accuracy was within ±2 ppm, and retention time reproducibility was excellent (RSD 0.13-0.74%). Sensitivity was high, with limits of detection at ≤1.0 ng/mL. Calibration curves were linear (R^2 ≥0.998), and intra- and inter-run precision showed minimal variability (≤2.0% CV). Matrix effects were well controlled, with over 85% of analytes within ±25%. Multi-energy fragmentation produced reproducible,

class-specific patterns across nitazene and orphine analogs, improving confidence in non-targeted identification.

Discussion: This study presents a rapid, non-targeted LC-HRMS workflow for detecting nitazene and orphine-type opioids in urine, addressing a critical gap in toxicological screening. The ability to resolve 19 of 20 nitazene isomers within an ultra-fast runtime, combined with systematic MS/MS characterization, represents a significant advancement in analytical performance. Importantly, the non-targeted design enables adaptability to emerging analogs, a key requirement in the evolving drug landscape. This approach offers a scalable solution for forensic and clinical laboratories, with important implications for early detection, improved analytical confidence, and strengthened public health responses to emerging synthetic opioid threats.

Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

O-140

Investigation of Methylenedioxynitazene Metabolism in Human Hepatocytes: Identification of Metabolic Biomarkers Using LC-HRMS/MS

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Abstract

Introduction: The continuous emergence of nitazene analogues has substantially contributed to the increasing number of fatalities associated with new synthetic opioids (NSOs). Among these compounds, methylenedioxynitazene, first identified on the illicit drug market in April 2024, represents a newly emerging benzimidazole opioid for which pharmacological activity, potency, and metabolic fate remain largely uncharacterized. The lack of metabolic data significantly limits toxicological detection and interpretation in both clinical and forensic contexts. Therefore, the identification of specific metabolic biomarkers is essential to document exposure, particularly when parent compounds undergo rapid biotransformation or are present at low concentrations in biological specimens.

Objectives: This study aimed to investigate the *in vitro* metabolism of methylenedioxynitazene using pooled human hepatocytes, characterize its phase I and phase II metabolites by liquid chromatography–high-resolution tandem mass spectrometry (LC-HRMS/MS), and identify potential biomarkers of methylenedioxynitazene exposure to improve toxicological detection in forensic and clinical settings.

Methods: Methylenedioxynitazene standard was incubated for 3h with 10^6 pooled human hepatocytes/mL in Williams' Medium E supplemented with 20 mmol/L HEPES buffer. After incubation, samples were analyzed by LC-HRMS/MS. Chromatographic separation was performed using a biphenyl-bonded analytical column (150 × 2.1 mm, 2.6 µm). The LC system was coupled to a Q Exactive Orbitrap mass spectrometer (Thermo Scientific) operating in full-scan acquisition and data-dependent MS/MS mode. *In vitro* metabolism was characterized in human hepatocyte incubation with *in silico* prediction (GLORYx), LC-HRMS/MS analysis, and Compound Discoverer software-assisted targeted/untargeted data mining.

Results: The LC-HRMS peak area of methylenedioxynitazene decreased by 35% after 3h of incubation compared with 0h under the same experimental conditions. A total of 19 metabolites were identified after 3h, indicating extensive phase I and phase II biotransformation. Methylenedioxynitazene remained the most abundant analyte, while the most intense phase I metabolite resulted from *N*-deethylation. Other abundant metabolites were generated through ring opening of the methylenedioxyphenyl moiety, nitro reduction, hydroxylation, and oxidative deamination. Phase II biotransformation involved *O*-glucuronidation and sulfation, with two *O*-glucuronidated and two sulfated metabolites identified. Notably, nitro reduction followed by acetylation represented a relevant secondary pathway, highlighting the nitro group as an important site of metabolic transformation. Overall, the metabolic pattern suggests that both oxidative and conjugated metabolites may represent potential analytical targets for toxicological screening.

Discussion: Methylenedioxynitazene undergoes extensive metabolism in human hepatocytes, with *N*-deethylation representing the predominant phase I pathway and the *N*-deethylated metabolite emerging as the most promising biomarker of exposure. Additional relevant transformations included ring opening of the methylenedioxyphenyl moiety, nitro reduction, and hydroxylation, which may contribute to extending the detection window in authentic toxicological specimens. The identification of phase II metabolites further supports the importance of including both unconjugated and conjugated metabolites in analytical screening strategies. These findings provide the first comprehensive characterization of methylenedioxynitazene metabolism and establish a valuable basis for future pharmacokinetic investigations, method development, and interpretation of intoxication cases involving this newly emerging nitazene analogue.

Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

O-141

Fatal Intoxication with the Nitazene *N*-Pyrrolidino Fluetonitazene: Quantification, Metabolism and μ-Opioid Receptor Affinity

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Abstract

Introduction: The new synthetic opioid *N*-pyrrolidino-fluetonitazene (N-pyrrolidino-4'-(2-fluoroethoxy) nitazene) was first detected in the European Union in 2024. It is the fluorinated analogue of etonitazepyne and was first developed and studied in the 1950s-60s by the Swiss pharmaceutical company Chemische Industrie Basel (CIBA). Nitazenes can interact with opioid receptors, including μ- (MOR), δ- (DOR), κ- (KOR) and nociception (NOP). In the forensic context, MOR is the particularly since it mediates opioid-induced respiratory depression.

Objectives: This study investigated the *in vivo* and *in vitro* metabolism of *N*-pyrrolidino fluetonitazene based on post-mortem samples (femoral blood, cardiac blood and urine) from a fatal intoxication and pooled human liver microsomes (pHLM), and tested its MOR affinity.

Methods: Metabolite elucidation was conducted using liquid chromatography-high resolution tandem mass spectrometry (LC-QTof) and quantification of the case samples was performed using liquid chromatography-tandem mass spectrometry (LC-MS/MS). Furthermore, we tested the MOR affinity of the nitazene using a novel LC-MS/MS-based MOR affinity assay.

Results: Eight different *in vitro* metabolites were identified, of which three (M2 O-dealkylation, M6 oxidative deamination and M8 carboxylation to N-butanoic acid) could also be confirmed *in vivo* in urine. In blood, the phase II metabolite M9 was found. Biotransformations observed were dehydrogenation, O-dealkylation, oxidation, *N*,*N*-bisdealkylation, carboxylation, deamination and their combinations thereof. The parent compound was quantified using LC-MS/MS and LC-QTof in the two blood samples.

In post-mortem samples, the following compounds were quantified: *N*-pyrrolidino-fluetonitazene, (0.71 ng/mL), Δ^9-tetrahydrocannabinol (<0.75 ng/mL) and its hydroxy and carboxy metabolites (1.9 ng/mL and 23 ng/mL), citalopram (340 ng/mL) with its desalkyl-metabolite (140 ng/mL), lithium (1670 ng/mL) and blood alcohol (0.55 g/kg).

Compared to the reference compound fentanyl (K_i=3.09 nM), which was taken along on every assay plate as a quality control, *N*-pyrrolidino-fluetonitazene depicted a MOR affinity almost twenty times stronger (K_i=0.166 nM).

Discussion: *In vitro*, the most abundant metabolites were M1-M3 formed by dehydrogenation, O-dealkylation and their combination. *In vivo*, M9, formed by *N*-acetylation of 5-aminofluetodesnitazene, was the most abundant metabolite.

The quantitative analysis of the post-mortem samples revealed a nitazene-related opioid intoxication accompanied by medicinal drugs, alcohol and cannabinoids. The result of the determination of THC and its metabolites supports a non-recent cannabis consumption. The blood alcohol concentration of 0.55 g/kg (approx. 0.052 g/dL) is compatible with a mild alcohol intoxication. Both the citalopram and lithium concentration were in therapeutic concentrations. The *N*-pyrrolidino-fluetonitazene concentrations align with results reported from Kriikku et al. who found a median concentration of 1.3 ng/mL in five *N*-pyrrolidino-fluetonitazene intoxications.

A recently presented LC-MS/MS-based MOR affinity assay was applied in this study. It overcomes limitations of radioligand-based assays such as radioactive waste, relatively long data acquisition times, higher operational costs and the need for specialized licensing and regulatory compliance. The result of the MOR assay aligned reasonably well with radioligand data in the literature for both *N*-pyrollidino-fluetonitazene and the reference compound fentanyl.

For the confirmation of a *N*-pyrrolidino-fluetonitazene intoxication, we recommend the *N*-pyrrolidino-fluetonitazene specific metabolites M8 and M9 as a marker metabolites.

Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

Conflict of Interest

This research was in whole or in part funded by a Swiss National Science Foundation project grant.

O-142

Small Change, Big Difference? In Vitro Characterization of a Series of 5-Cyano Desnitazene Opioids or "Cyanazenes"

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Abstract

Introduction: Recently, the new synthetic opioid (NSO) market has shifted from fentanyl derivatives toward highly potent nitazenes, which now represent one of the most prevalent NSO classes. This ongoing emergence of highly potent variants, as standalone products or as adulterants in street drugs, emphasizes the importance of rapid detection and pharmacological evaluation to support effective risk assessment. One of the most recent nitazenes to be identified on European markets is 5-cyano isotodesnitazene, a variant of isotonitazene, in which the 5-nitro substituent is replaced by a 5-cyano group.

Objectives: Using our in-house *in vitro* μ-opioid receptor (MOR) activation assay, we set out to evaluate the MOR activation potential i.e., efficacy and potency, of a series of 5-cyano nitazenes (also coined "cyanazenes"), including 5-cyano isotodesnitazene, 5-cyano protodesnitazene, 5-cyano etodesnitazene and 5-cyano metodesnitazene, compared to structurally relevant comparators (i.e. 5-nitro substituted analogues and des-analogues lacking a 5-substituent). In addition, the MOR activation potential of an authentic powder of 5-cyano isotodesnitazene was assessed. To support fast detection and harm reduction, two versions (1.0 and 2.0) of immunoassay nitazene test strips (NTS) were evaluated for their ability to detect the new cyanazene variants.

Methods: MOR activation was assessed via luminescence monitoring, using a live-cell based assay, in which the recruitment of β-arrestin 2 to the activated MOR results in functional complementation of a split-nanoluciferase (NanoBiT® principle). All compounds (including the powder) were tested in a concentration range from 1 pM to 10 μM. NTS analysis was performed using BTNX Rapid response NTS versions 1.0 and 2.0. Compounds yielding a positive result at 3000 ng/ml were subsequently tested at 1000 ng/ml, whereas those with a negative result were tested at 9000 ng/ml.

Results: Among the cyanazenes evaluated, 5-cyano etodesnitazene exhibited the highest potency (EC50 = 32.4 nM), while 5-cyano isotodesnitazene (EC50 = 71.5 nM) and 5-cyano protodesnitazene (EC50 = 73.7 nM) showed comparable potency. 5-Cyano metodesnitazene (EC50 = 373 nM) demonstrated the lowest potency of the four tested cyanazenes. Structure-activity relationships revealed consistent trends: all cyanazenes showed reduced activity relative to their 5-nitro analogues and displayed activities comparable to their respective des-analogues. Moreover, when compared to fentanyl, all evaluated cyanazenes were substantially less potent. The potency of the authentic powder of 5-cyano isotodesnitazene was somewhat lower but in the same order of magnitude compared to the 5-cyano isotodesnitazene reference standard. NTS analysis demonstrated improved sensitivity of the 2.0 NTS, which detected all cyanazenes and isotodesnitazene at 3000 ng/ml, whereas the 1.0 NTS failed to detect isotodesnitazene and 5-cyano protodesnitazene at 3000 ng/ml.

Discussion: *In vitro* characterization of four newly emerging cyanazenes allowed to determine relevant structure-activity relationships, highlighting the critical role of the 5-nitro group in the MOR activation potential of nitazenes, as the results indicate that 5-cyano substitution does not compensate for the potency loss that occurs upon removal of the 5-nitro group. Moreover, NTS analysis suggests that the 2.0 NTS exhibit improved cross-reactivity, detecting both cyanazenes and isotodesnitazene. Together, these findings provide critical insights that can support early risk assessment and harm reduction strategies.

Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

O-143

Inhibition of Cardiac Voltage-Gated Ion Channels by New Synthetic Opioids

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Abstract

Introduction: The cardiac voltage gated potassium channels K_V11.1 (also known as human ether-a-go-go; hERG) and K_V7.1/KCNE1 mediate the repolarizing current of the cardiac action potential which helps coordinate the heartbeat. Inhibition of these channels can lead to adverse cardiovascular effects, including QT prolongation, arrhythmias, and sudden cardiac death. Some opioids (e.g., methadone, fentanyl) have been found to inhibit hERG function, which suggests that some new synthetic opioids (NSOs) like the nitazenes or orphines may also interact with hERG and/or K_V7.1/KCNE1, thereby producing cardiotoxic effects.

Objectives: This study aimed to evaluate the cardiotoxic potential of NSOs through interaction with hERG and K_V7.1/KCNE1.

Methods: hERG and K_V7.1/KCNE1 were stably expressed in Chinese hamster ovary cells (B'SYS GmbH). Recordings were performed using the QPatch, an automated patch clamp system (Sophion Biosciences A/S). A step voltage protocol was applied and currents were recorded. The voltage protocol was recorded three times in vehicle solution (blank) to generate a stable baseline followed by five increasing concentrations of the compound, then another vehicle to test recovery.

Results: All of the 19 NSOs tested, including 6 nitazenes, 11 orphines, and 2 methadone analogs, inhibited the maximal conductance (G_{max}) of hERG except 5,6-dichloro brorphine. The IC_{50} values of the nitazenes ranged from 0.15-0.63 μM; the IC_{50} values of the orphines ranged from 0.099-5.5 μM; and methiodone and N-piperidinyl methiodone had IC_{50} values of 52.6 μM and 12.5 μM, respectively, whereas methadone had an IC_{50} of 5.1 μM. None of the opioids tested affected the voltage dependence of activation (V½) of hERG.

N-desethyl-isotonitazene, the major metabolite of isotonitazene, also exhibited strong hERG inhibition (IC_{50} = 0.63 μM), although it was significantly less potent than isotonitazene (IC_{50} = 0.15 μM). Examination of the structure-activity relationships of the orphines demonstrated an increase in hERG G_{max} inhibition as the halogen increased in atomic radius. Iodorphine with an iodine was the most potent hERG inhibitor of the NSOs with an IC_{50} of 0.099 μM and was significantly more potent than brorphine with a bromine (IC_{50} = 0.29 μM), chlorphine with a chlorine (IC_{50} = 0.61 μM), fluorphine with a fluorine (IC_{50} = 2.8 μM), and orphine with a hydrogen (IC_{50} = 5.5 μM).

Most of the opioids did not inhibit the G_{max} of K_V7.1/KCNE1, except protonitazene and N-piperidinyl methiodone, and none affected the V½.

Discussion: This study demonstrates that most NSOs pose cardiotoxic risks due to strong hERG inhibition. Although N-piperidinyl methiodone and methiodone demonstrated weaker hERG inhibition than methadone, it is still possible that they could pose cardiotoxic risks given their reduced μ opioid receptor activity. In addition, the strong hERG inhibition of the metabolite

N-desethyl-isotonitazene demonstrates that metabolites may also contribute to the cardiotoxic risk of opioids. Evaluation of the structure-activity relationships at hERG may help with prediction of the cardiotoxic risk of newly emerging NSOs in the future. Overall, this study demonstrates the importance of evaluating the interaction of NSOs and their metabolites at cardiac voltage-gated potassium channels to inform their cardiotoxic potential.

Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

O-144

Comprehensive Metabolic Characterization and Enantioselective Profiling of *N*-Propionitrile Chlorphine, an Emerging Orphine-Type Synthetic Opioid

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Abstract

Introduction: During the last decade, new synthetic opioids (NSOs), especially potent 2-benzylbenzimidazole, represented a growing challenge for public health, clinical toxicology, and forensic laboratories. Following increasing international control measures and regulatory scheduling of nitazenes, the illicit market has shifted toward structurally modified analogs and alternative opioid scaffolds, including the benzimidazol-2-ones, also called Orphines. Among these novel compounds, *N*-propionitrile chlorphine has recently appeared on the illicit market as a structurally modified orphine-type opioid, characterized by the introduction of a nitrile moiety that may influence both its pharmacological properties and metabolic behavior. Despite its emerging relevance, data regarding its biotransformation pathways, stereoselective metabolism, and enantiomeric disposition remain extremely limited. The identification of metabolic pathways and stereoselective biotransformations of synthetic opioids is essential for toxicological interpretation and biomarker discovery. *N*-propionitrile chlorphine is a structurally modified compound for which metabolic fate and enantiomeric behavior remain insufficiently characterized.

Objectives: This study aimed to characterize the *in vitro* metabolism of *N*-propionitrile chlorphine in human hepatocytes, structurally elucidate metabolites using LC-Q Exactive Orbitrap HRMS/MS, and investigate enantioselective profiles of the parent drug and major metabolites using chiral HPLC-MS/MS.

Methods: *N*-propionitrile chlorphine was incubated for 3h with 10-donor pooled human hepatocytes. Incubates were analyzed by LC-HRMS/MS with a separation on a biphenyl-bonded analytical column and detection in full-scan MS and data-dependent MS/MS acquisition. Raw data were screened with Compound Discoverer. Analyses were supported by metabolite predictions in humans with GLORYx open-access software. Enantioselective analysis was performed using an HPLC-MS/MS. A chiral amylose-based stationary phase (Amylose-1) was employed under isocratic conditions using acetonitrile with 0.1% NH_4OH and water with 0.1% formic acid (90:10, *v/v*) at 1.0 mL/min.

Results: A total of 28 metabolites were identified, indicating phase I and II biotransformations. Metabolism was dominated by multi-site *N*-dealkylation, hydroxylation, oxidation, and secondary transformations, including carbonyl reduction, methylation, *N*-acetylation, and *O*-glucuronidation. The parent compound represented the most abundant signal, while the major metabolites were hydroxylated derivatives, followed by the dihydro metabolite and the *N*-dealkylated metabolite. Multiple metabolites resulted

from *N*-dealkylation of either the propanenitrile moiety (*N*-dealkylation-1) or the 1-chloro-4-ethylbenzene group (*N*-dealkylation-2), suggesting both sites as major metabolic sites. Two *O*-glucuronidated metabolites were also detected.

Chiral LC-MS/MS analysis under the selected enantioselective conditions provided near-baseline separation of the parent compound, and baseline separation of metabolites produced after hydroxylation, *N*-dealkylation-1 and a combination of *N*-Dealkylation-1, *N*-Acetylation and Carbonyl Reduction). In contrast, no enantioseparation was observed for the metabolites produced after di-hydroxylation and *N*-dealkylation-2 and hydroxylation.

Discussion: *N*-propionitrile chlorphine undergoes extensive metabolism in human hepatocytes, with *N*-dealkylation and hydroxylation representing the dominant pathways. The presence of multiple metabolic sites and several secondary biotransformations suggests complex metabolic behavior relevant for toxicological screening and biomarker selection. An amylose-based chiral stationary phase allowed the enantioseparation of selected metabolites and the parent compound under the applied chromatographic conditions, providing a basis for future investigations on the potential enantioselectivity of metabolism in authentic samples. These findings highlight the importance of incorporating enantioselective analytical strategies in metabolic studies of synthetic opioids. The developed HPLC-MS/MS method provides a robust platform for enantioselective metabolite profiling, supporting future pharmacokinetic, toxicological, and forensic investigations.

Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

O-145

Phase I Metabolism Study on the New Synthetic Opioid Methiodone Involved in Seven Intoxication Cases

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Abstract

Introduction: In recent years, novel psychoactive substances (NPS) such as new synthetic opioids have raised particular concerns due to their high potency, unpredictable toxicity, and association with a rising number of fatal cases. A remarkable trend among these substances is the reemergence of compounds initially developed by pharmaceutical companies in the 1950s and 1960s. Methiodone, an analog of methadone, exemplifies this phenomenon. Although originally synthesized decades ago, it has recently been involved in more than 40 cases investigated in Freiburg, Germany, demonstrating its high relevance. Due to its unknown metabolic pathways, insights into methiodone's biotransformation are essential.

Objectives: This study investigates seven cases involving this opioid, analyzing its phase I metabolism using a pooled human liver microsomes (pHLM) assay and human biosamples.

Methods: In vitro metabolism studies were conducted with pHLM. Additionally, human samples from six post-mortem cases (covering urine, cardiac and femoral blood, liver homogenate) and a clinical intoxication case (analysis of urine and serum) were examined. Major metabolites were tentatively identified by liquid chromatography-quadrupole time-of-flight mass spectrometry (LC-QTOF-MS, mass accuracy ≤ 10 ppm), while minor metabolites were characterized by targeted screening with liquid chromatography-tandem mass spectrometry (LC-MS/MS). Methiodone concentrations in blood samples were quantified using LC-MS/MS.

Results: Methiodone was detected in all analyzed biological matrices in the fatal cases, but only in urine of the intoxication case. Concentrations ranged from 25 to 930 ng/mL in blood samples. Two major phase I metabolites were tentatively identified, involving *N*-dealkylation (M1) and *N*,*N*-di-dealkylation (M2). In addition, minor metabolic pathways involving hydroxylation (M3-M5), two-fold hydroxylation (M6) and combinations of *N*-dealkylation and hydroxylation (M7-M9) were observed solely in vivo. Notably, a metabolic reaction involving ring closure, analogous to the biotransformation of methadone to EDDP, was not observed. Both M1 and M2 could be found in vitro and in all investigated matrices of the fatal cases. In the intoxication case, M1 and M2 were detected in urine, but only M1 was found in blood. No other metabolites were present in the blood samples. M9 was consistently found in all investigated urine and liver samples, while M7 and M4 were found in a majority of them. Other metabolites were detected sporadically in urine and/or liver samples.

Discussion: The primary metabolic pathway of methiodone is *N*-dealkylation, leading to the formation of M1 and M2. Importantly, even if the parent is not detected in blood, the major metabolite M1 may still be present. Therefore, M1 appears to have a longer detection window in blood than the parent. The in vitro model predicted only the major metabolites, M1 and M2. In conclusion, analysis should prioritize the parent compound, as well as the metabolites *N*-dealkyl methiodone (M1), *N*,*N*-di-dealkyl methiodone (M2) and *N*,*N*-di-dealkylhydroxy methiodone (M9).

Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

O-146

Navigating the Evolving Novel Synthetic Opioid Landscape

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Abstract

Introduction: The detection of novel synthetic opioids (NSOs) has remained prevalent in forensic toxicology laboratories. Historically, fentanyl and its analogs (fentalogues) have been the dominant NSOs, but recently the emergence of nitazenes and orphines has posed a significant challenge. In late 2025, the Ohio Bureau of Criminal Investigation (BCI) first identified cychlorphine in Southwest Ohio and according to the Center for Forensic Science Research and Education (CFSRE), the positivity rate of this compound has increased nationally since mid-2025. This identification in Ohio, along with public health alerts and drug trend reporting, prompted a comprehensive reassessment of the NSOs scope of testing.

Objectives: To proactively advance the existing fentanyl assay by (1) removing fentalogues no longer (or never) detected in the seized drug supply and (2) combining fentalogues with emerging nitazenes and orphines into one consolidated UHPLC-MS/MS panel.

Methods: A retrospective review (2021-2025) of fentanyl positive cases was conducted to guide panel refinement. Infrequently reported compounds in the previous assay were excluded, with the exception of carfentanil. Literature review and surveillance data guided the selection of new analytes to include in the method. An updated analytical method was subsequently performed using an ultra-high performance Thermo Fisher Scientific™ Vanquish™ Horizon liquid chromatography system coupled with a Thermo Fisher Scientific™ TSQ Altis Plus™ triple quadrupole tandem mass spectrometer (UHPLC-MS/MS) using a Kinetex® 2.6 µm Biphenyl 100 Å LC column 150 x 2.1 mm. Mobile phases consisted of 0.1% formic acid and 5 mM ammonium acetate in LC-MS grade water and 0.1% formic acid in methanol.

Results: Of the compounds in the former assay, 19 analytes were removed due to negligible or absent detection in casework over the last five years and 14 analytes remained. The finalized panel will consist of nearly 70 compounds: fentanyl/fentalogues, nitazenes, orphines, and adulterants. A consequence of developing a panel of this size is the existence of multiple isobaric/isomeric groups; this is a profoundly challenging aspect in method development as these compounds may share the same transition ions and could potentially coelute. Of the 18 isobaric/isomeric groups, 17 were successfully resolved except for *meta*- and *para*-chlorofentanyl. In addition to the broadened scope of the updated assay, sample volume was reduced, and dynamic range was expanded.

Discussion: Forensic toxicology methods must be continuously updated based on research to remain relevant amid changing drug trends. Unlike traditional drugs of abuse where established panels require infrequent updating, the NSOs landscape is constantly evolving. This requires an approach that is more sensitive to real-time surveillance data from public health and research institutions. The decision to consolidate the fentalogues, nitazenes, orphines and adulterants was driven by their co-occurrence in casework. There are current limitations with screening for nitazenes and orphines; however, using case history for postmortem cases and combining the analytes into one panel could lead to fewer false negative results.

Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

O-147

Can Molecular Docking and Dynamics Predict Opioids Potency? Pharmacological and Computational Insights From Methylenedioxynitazene

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Abstract

Introduction: Novel synthetic opioids (NSOs) have emerged as a major public health and forensic toxicology concern due to their high potency and increasing involvement in fatal intoxications. Currently, nitazenes represent one of the most frequently identified subclasses of NSOs, often associated with severe intoxications and fatal overdoses. Rapid toxicological assessment of emerging NSOs remains challenging, as pharmacological data are often unavailable at the time of first forensic detection. Methylenedioxynitazene is a newly identified nitazene analogue for which little to no pharmacological or computational characterization has been reported. In this context, computational approaches such as molecular docking and molecular dynamics (MD) simulations may represent valuable tools for the early evaluation of emerging opioids.

Objectives: This study aims to integrate analytical, pharmacological, and computational approaches, and highlight the role of molecular docking and dynamics as an early predictive strategy for assessing the pharmacological relevance of emerging NSOs. Chemical synthesis and analytical characterization of methylenedioxynitazene and its 5-amino metabolite were performed to evaluate their MOR agonist activity *in vitro* and investigate their molecular binding patterns through computational modeling.

Methods: Methylenedioxynitazene and its putative 5-amino metabolite were synthesized to obtain analytical standards. Structures were confirmed by NMR (^{1}H and ^{13}C), ATR-FTIR, LC-HRMS/MS and GC-MS/MS, allowing the identification of diagnostic fragments. MOR activation was evaluated using an HTRF-based GTP-G_i binding assay, measuring potency (EC_{50}) and efficacy (E_{max}). Computational analysis was conducted using molecular docking on the MOR-crystal structure (PDB: 5C1M), followed by 500 ns MD simulations to assess ligand accommodation, conformational stability, and interaction persistence within the receptor binding pocket.

Results: Chemical synthesis produced compounds with high purity, and analytical characterization established their analytical profiles. *In vitro* pharmacology demonstrated that methylenedioxynitazene is a potent MOR-agonist (EC_{50}=102 nM; E_{max}=120%), showing lower potency but higher efficacy than Fentanyl (EC_{50}= 2.5 nM; E_{max}=100%). Conversely, the 5-amino metabolite displayed significantly reduced activity (EC_{50}=502 nM; E_{max}=29%), indicating weak partial agonism. Docking studies showed higher binding affinity for methylenedioxynitazene (–10.19 kcal/mol) compared to its metabolite (–9.12 kcal/mol). MD simulations revealed rapid and stable accommodation of methylenedioxynitazene within the MOR binding pocket, whereas the

metabolite exhibited delayed stabilization and unstable conformational behavior. As confirmed by published *in vitro* receptor study, the nitro group was identified as a structural determinant, essential for proper receptor binding orientation and activation.

Discussion: This study provides the first comprehensive characterization of methylenedioxynitazene through an integrated multidisciplinary approach. The pharmacological and computational findings demonstrate that methylenedioxynitazene is a potent and highly efficacious MOR-agonist, supporting its classification as a high-risk emerging nitazene opioid. The marked reduction in activity of the 5-amino metabolite suggests that nitro-group reduction represents a relevant metabolic deactivation pathway. Computational predictions highlight molecular docking and dynamics as a valuable strategy for toxicological assessment of emerging opioids analogues. Docking provided the key aminoacids involved in the interactions, while MD offered a mechanistic insight of receptor binding determinants and accurately predicted the activity loss associated with nitro-group reduction. These findings support computational analysis as a predictive tool for new NSOs, demonstrating how docking-driven strategies improve characterization of emerging NSOs and strengthen forensic identification and public health risk evaluation.

Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

O-148

Metabolism Study and Quantification of the New Synthetic Opioid Methiodone in Clinical and Forensic Cases via LC-MS/MS

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Abstract

Introduction: The increased prevalence of new synthetic opioids over the past decade has become a major challenge in global healthcare and has been associated with a rising number of unintentional overdoses and drug-induced deaths. One of those synthetic opioids is methiodone, which was initially developed in 1948. Structurally, methiodone can be considered a derivative of methadone displaying a bioisosteric sulfone group replacing the carbonyl moiety. To the best of our knowledge, there are no clinical or postmortem-toxicological data published for methiodone so far.

Objectives: One non-fatal and four fatal poisoning cases involving methiodone are presented. Two were clinical cases, one of which later became a forensic postmortem case; the other three were forensic postmortem cases. A liquid chromatography-tandem mass spectrometry (LC-MS/MS) method was developed for determination of methiodone in serum and femoral blood and validated using a reduced validation approach for single case studies [1]. Additionally, preliminary metabolism studies were performed using the fungus *Cunninghamella elegans*, an established model for mammalian phase I metabolism.

Methods: Routine toxicological analysis was performed in all cases. For quantitative analysis of methiodone, 25 µL of serum or femoral blood were precipitated with acetonitrile/methanol 4:1 (v:v), diluted and subsequently analyzed by LC-MS/MS analysis on a Q-Exactive™ Focus instrument. Chromatographic separation was performed on an EC100/3 Nucleoshell RP 18 plus column (100 mm x 2.1, 2.7 µm, Macherey Nagel). The MS was operated in positive ESI and parallel reaction monitoring mode. For metabolism studies, *Cunninghamella elegans* was cultivated in 9 mL Sabouraud glucose medium. After three days 1 mL of 1 mM methiodone hydrochloride was added and incubated for four days (26 °C). Thereafter, 100 µL of supernatant were analyzed using a LC-MS/MS-based routine urine screening approach [2].

Results: Methiodone was detected alongside multiple other drugs in all five cases. No interferences with the detection of methiodone were observed in the applied LC-MS/MS method. Limit of detection, lower limit of quantification and linearity range were 10 ng/mL, 50 ng/mL, and 50–1200 ng/mL, respectively, in both serum and femoral blood. Accuracy, precision and matrix effects were within acceptance ranges. Methiodone concentrations in serum orfemoral blood [ng/mL] were 650 (case 1, non-fatal), 77 (case 2, fatal), 1100 (case 3, fatal), 920 (case 4, fatal), and 1000 (case 5, fatal). Metabolic profiling using *Cunninghamella elegans* identified nor-methiodone as the principal metabolite followed by hydroxy-methiodone. Results from analysis of the authentic urine specimens further indicated bis-nor-methiodone, nor-hydroxy-methiodone, methiodone-*N*-oxide, and phase II conjugates as additional metabolites.

Discussion: These findings provide the first clinical and forensic toxicological characterization of methiodone, demonstrate its toxicological relevance, and support its inclusion in routine toxicological screening. Although the presented cases were multiple-drug overdoses, methiodone was considered the primary or at least contributary source of toxicity in all cases. The clinical cases showed typical symptomatology of opioid overdoses necessitating medical support. The wide concentration range of methiodone indicates considerable inter-individual differences of methiodone toxicity as described for methadone and other opioids.

[1] F.T. Peters et al. Forensic Sci Int 165(2-3) (2007) 216-24.

[2] D.K. Wissenbach et al. Anal Bioanal Chem 400(10) (2011) 3481-9.

Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

O-149

Comprehensive LC-MS/MS Analysis of Synthetic Cocaine Derivatives: From Stereoisomer Differentiation to Real-World Forensic Applications

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Abstract

Introduction: The rapid emergence of new psychoactive substances has led to a growing market for synthetic cocaine derivatives, including phenyltropanes, 1,4-dialkylpiperazines, phenylpiperidines, benztropines, and dimethocaine. Compounds such as troparil, dichloropane, and dimethocaine are widely distributed via online “research chemical” platforms, with newer substances such as DMNPC (N,O-dimethyl-4β-(2-naphthyl)piperidin-3β-carboxylate) recently reported. These compounds mimic the tropane scaffold of cocaine, with (–)-2β,3β stereoisomers generally exhibiting the highest psychoactive potency. Their detection in forensic and traffic medicine remains limited due to the lack of validated analytical methods capable of resolving structurally similar and stereochemically distinct compounds.

Objectives: This study aimed (i) to develop and fully validate a comprehensive LC-MS/MS multi-analyte method for the detection and quantification of cocaine and a broad spectrum of synthetic cocaine derivatives in biological matrices, and (ii) to establish stereoisomer-resolving analytical strategies and apply these methods to authentic biological and seized samples, including metabolic investigations.

Methods: A diverse panel of synthetic cocaine derivatives and their stereoisomers was synthesized as reference material. A multi-target LC-MS/MS method was developed for blood, serum, and urine and validated according to national forensic guidelines. Analyses were performed on a Raptor Biphenyl column (150 × 2.1 mm, 5 µm) using a gradient with 2 mM ammonium formate with 0.1% formic acid in water (A) and acetonitrile containing 0.1% formic acid (B). In addition, dedicated analytical approaches were implemented to differentiate stereoisomers of key compounds such as troparil and dichloropane. The method was applied to authentic blood and urine samples as well as to substances obtained from online vendors.

Results: The validated method enabled selective, sensitive, and reliable quantification of cocaine and multiple synthetic derivatives in biological matrices in pharmacologically relevant concentration ranges. Validation criteria, including selectivity, linearity, and intra- and inter-day precision, were fulfilled for the majority of analytes. Application to authentic samples confirmed the presence and quantification of troparil, dichloropane, and their metabolites in blood and urine. Analysis of vendor samples revealed frequent mislabeling, particularly among phenyltropanes. Notably, in the seized samples only enantiomeric mixtures of the less psychoactive 2β,3α stereoisomers were detected.

Discussion: This work presents the first comprehensive and fully validated LC-MS/MS multi-method for the broad-spectrum detection and quantification of synthetic cocaine derivatives in biological matrices and additional dedicated analytical methods for the stereochemical characterization, as demonstrated here for compounds such as troparil and dichloropane. The findings demonstrate that stereochemical resolution is essential for accurate pharmacological and toxicological risk assessment, as structurally similar compounds can differ markedly

in psychoactive potency. Furthermore, the observed mislabeling of commercially available substances highlights a significant and previously underrecognized challenge in forensic casework. These results underline the necessity of advanced analytical approaches to ensure reliable substance identification and interpretation in both clinical and forensic toxicology. Future work will focus on the continuous expansion of the method to include newly emerging derivatives, thereby strengthening its applicability in cases of suspected drug misuse and abstinence monitoring.

Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

Conflict of Interest

Grant/research support from the Deutsche Forschungsgemeinschaft (DFG) SU-1266/1-1; no other conflicts declared.

O-150

Detection of N-propionitrile Chlorphine (Cychlorphine) Throughout the United States in Healthcare Patient Urine Samples

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Abstract

Introduction: N-propionitrile chlorphine (cychlorphine), a synthetic opioid derivative of brorphine, has recently emerged as a concern in clinical and forensic toxicology, as well as in the general media, due to its identification in multiple overdose deaths, reported potent pharmacological activity and limited data regarding its prevalence. It is typically observed with other novel psychoactive substances and illicit compounds; however, little other information exists on prevalence of this novel opioid, particularly in pain patient and/or behavioral health patient populations that may be more susceptible to substance misuse. Timely detection is crucial, especially as many patients may not be aware of what they are consuming when engaging in drug use.

Objectives: Upon attending the presentation, the attendee will be able to describe the prevalence of urine cychlorphine in chronic pain and behavioral health populations, as well as information regarding demographic spread and co-occurring substance detection. This presentation will guide the learner to consider the possibility of cychlorphine presence when conducting forensic or clinical evaluations.

Methods: This study was IRB approved. Cychlorphine was analyzed in the laboratory as part of a larger Novel Psychoactive Substances (NPS) panel for which target analytes were chosen based on information from the Center for Forensic Science Research and Education (CFSRE), US Drug Enforcement Administration and other online resources. Prior to analysis, urine samples were hydrolyzed and diluted with buffer. Samples were injected onto an Agilent 1290/Ultivo liquid chromatography/tandem mass spectrometry (LC-MS/MS) instrument; detection was via dynamic MRM analysis following positive electrospray ionization. Analytes were chromatographically separated on a biphenyl column. Upon request by a provider, samples were analyzed for any NPS compound (or drug class) during the study period of October 1, 2025 – March 30, 2026 and all samples meeting qualitative acceptance criteria for cychlorphine were included in this analysis.

Results: Of 471,236 samples evaluated, 167 (0.04%) samples from 119 patients contained detectable cychlorphine. Samples were limited to those submitted for NPS testing. Per month, detection rates remained generally consistent during the study period. In samples submitted, the largest rates (N) of detection were observed in MD (123), TN (10), IL (9), MA (6) and ME (6). Ages (N, %) and gender distributions were: 18-25 years (2, 1.2%), 26-40 years (68, 40.7%), 41-56 years (58, 34.7%), and 57-75 years (39, 23.4%) with 57 (34.1%) female and 110 (65.9%) male samples. Of the positive samples, 154 (92%) were also positive for fentanyl/norfentanyl, 102 (61%) were positive for cocaine markers (benzoylecgonine), and 24 (14%) were positive for heroin markers (6-MAM). Several co-positive NPS were also detected (when ordered) with highest rates observed for xylazine/4-OH-xylazine (N=109, 65%), medetomidine/3-OH-medetomidine (N=34, 20%), tianeptine/tianeptine MC5 (N=31, 19%), and o-methylfentanyl (N=28, 17%).

Discussion: The detection of cychlorphine in urine samples from pain compliance and behavioral health patients highlights the need for ongoing surveillance of emerging NPS in these populations. The validated LC-MS/MS method offers a robust approach for monitoring cychlorphine exposure, supporting clinical decision-making and risk mitigation strategies. Further research is warranted to assess the clinical implications and long-term trends of cychlorphine use, particularly in combination with other novel psychoactive substances and/or illicit drugs.

Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

O-151

Integrated Clinical-Toxicological Surveillance of Psychoactive Substances in Open Drug Scenes in Sao Paulo, Brazil

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Abstract

Introduction: The rapid expansion of New Psychoactive Substances (NPS) has posed significant challenges to forensic and clinical toxicology due to their structural diversity, limited toxicological data, and continuous emergence of new analogues. In open drug scenes, characterized by social vulnerability and polysubstance use, these challenges are intensified and often not captured by traditional surveillance systems based on hospital reports. Integrating clinical and toxicological data within community-based settings represents a promising approach for early detection of emerging substances and exposure patterns.

Objectives: This study aimed to describe the clinical-epidemiological profile and toxicological findings of individuals from open drug scenes in Sao Paulo, Brazil, with emphasis on the detection of NPS and the feasibility of an integrated surveillance model.

Methods: A prospective observational study was conducted at a community-based harm reduction service (*HUB de Cuidados em Crack e Outras Drogas*, Sao Paulo, Brazil). A total of 194 adults were recruited during initial screening between June 2025 and April 2026 (CAAE No. 95857326.8.0000.5404). Sociodemographic characteristics, indicators of social vulnerability, self-reported substance use (including polysubstance use and “K drugs”), and clinical signs of acute intoxication were collected using a standardized questionnaire. Oral fluid samples were collected with the Quantisal® device and analyzed by liquid chromatography-tandem mass spectrometry (LC-MS/MS). Descriptive analyses integrating clinical, epidemiological, and toxicological data were performed.

Results: Participants were predominantly cisgender men (92.3%), with a mean age of 38 years (SD 9), most self-identifying as black or brown (62.9%) and experiencing homelessness (78.4%). Self-reported polysubstance use was highly prevalent, particularly involving cocaine (80.6%), crack (74.8%), and cannabis (55.5%). During the three months preceding the interview, 95.5% reported cocaine or crack use, and 90.2% reported daily consumption. Toxicological analysis detected at least one substance in all samples, with a mean of 10.08 substances per sample (median 10; range 3-21). Cocaine was detected in 99.5% of samples, followed by lidocaine (95.4%), diazepam (38.7%), venlafaxine (24.7%), and Δ9-THC (24.2%). Antidepressants, antipsychotics, benzodiazepines, and anticonvulsants were also frequently identified. Synthetic cannabinoid receptor agonists (SCRAs) were detected in

29% of samples, including MDMB-4en-PINACA, ADB-BINACA_ADB-BUTINACA, 5F-MDMB-PINACA, 4F-MDMB-BUTINACA, BZO-POXIZID, and MDMB-CHMINACA. Notably, 60.5% of SCRA-positive individuals denied recent use of “K drugs”. Clinical findings revealed signs of intoxication (27.3%), withdrawal (24.3%), suspected psychosis (16.8%), disorientation (28.6%), and a high prevalence of lifetime suicide attempts (42.2%).

Discussion: These findings demonstrate complex exposure patterns in open drug scenes, characterized by extensive polysubstance use, widespread cocaine adulteration with lidocaine, and the co-detection of pharmacologically diverse substances with potential clinical relevance. The detection of SCRAs confirms the circulation of NPS in this setting, while the discrepancy between self-reported use and toxicological findings highlights important limitations of surveillance systems based solely on self-report. The substantial burden of psychiatric morbidity further emphasizes the vulnerability of this population. Integrated clinical-toxicological surveillance approaches may improve the early detection of emerging substances and strengthen both public health and forensic monitoring strategies.

Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

O-152

Prevalence Rates, Concordance, and Cognitive Correlates of Biochemically-Verified Cannabis Use in a Large Cohort of Healthy Adolescents Over Time

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Abstract

Introduction: Adolescent substance use is a significant public health concern, though the scope of the problem is difficult to ascertain given reliance on self-reported substance use information. Recent, large cohort studies in adolescents and young adults suggest underreporting of substance use. The scale over time and clinical correlates of such underreporting are not currently known.

Objectives: Analyses here aim to determine concordance between self-reported substance use and biochemical verification through hair samples and to estimate U.S. prevalence of cannabis use. We further assess cognition over time in those who use cannabis, whether identified by self-report or toxicological testing, and use repeat hair cannabinoid testing to assess THC and CBD in relation to cognition.

Methods: Data came from the U.S., nationwide Adolescent Brain Cognitive Development Study (n=11,868; age 9-10 at Baseline, age 15-16 at Wave 6). Participants were followed annually, with data here from 2016 to 2024. Participants self-reported past 3-month substance use, completed biannual cognitive testing, and toxicological testing (urine, oral fluid, and hair). Following washing to mitigate environmental contamination, liquid chromatography and gas chromatography tandem mass spectrometry (LC or GC/MS-MS) were used to test hair samples for biochemically verified substance use, with results compared to self-reported substance use. When possible, models used THCCOOH to confirm personal ingestion. Multi-step weighting methods estimated national prevalence trends of THCCOOH over time, adjusting for discrepancies in sample representation due to recruitment demography, missed visits, and hair sample testing. Longitudinal mixed-effects modeling assessed trajectories of cognition between the cannabis use group (self-report or hair positive) and the non-use group in all participants. Follow-up analyses in a subsample examined whether cognitive trajectories differed by cannabinoid content in hair, with THC, CBD, and control groups.

Results: Concordance between self-report and toxicological data improved with age (for cannabis, ages 11-12=<1%; ages 15-16=45%). One third of those who used cannabis were initially identified solely through toxicological testing. Weighted estimates of biochemically verified substance use indicated 7.1% (95%CI: 6.0-8.3) of 15-16 year olds engaged in biochemically detected cannabis use. Those who used cannabis demonstrated restricted trajectories of cognitive growth over time, with small to large effect sizes across domains (immediate recall and delayed memory, processing speed, inhibitory control, visuospatial processing, language, and working memory; βs=-0.11- -0.52). THC in hair was linked

specifically to poorer episodic memory trajectories than in Controls (β=-0.60, p=.007), with no difference between CBD exposed and Controls.

Discussion: One out of three youth were identified as using cannabis through toxicological testing, with no corresponding self-reported use at that time. Concordance in reporting demonstrated improved biochemical verification with age. Biochemical verification reflects substantive cannabis use by ages 15-16, supporting combining toxicological and self-report data to improve identification of substance use in youth when possible. Through using both self-report and toxicological testing, results revealed those who used cannabis demonstrated plateaued trajectories of cognitive development over time. THC use may specifically relate to episodic memory performance over time during this developmental period, suggesting a potential mechanism for altered cognitive trajectories.

Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

O-153

Longitudinal Assessment of Alcohol Biomarkers for Differentiating Self-Reported Drinking Behaviors

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Abstract

Introduction: Alcohol remains one of the most widely used substances, but the rapid elimination of ethanol limits its utility as a direct biomarker beyond acute exposure. Although ethyl glucuronide (EtG) and ethyl sulfate (EtS) extend detection windows, phosphatidylethanol (PEth) homologues, such as PEth 16:0/18:1 (POPEth) and PEth 16:0/18:2 (PLPEth), have emerged as sensitive and specific biomarkers for characterizing drinking behaviors. However, real-world longitudinal data comparing these biomarkers across biological matrices and relating analytical findings to drinking behavior remain limited, thereby warranting further investigation.

Objectives: The goals of this study were to evaluate POPEth and PLPEth in relation to self-reported alcohol consumption, compare their performance with EtG and EtS as biomarkers of recent versus repeated or cumulative alcohol use, and assess whether these alcohol biomarker concentrations in blood, urine, and oral fluid could differentiate drinking behaviors and improve interpretive guidance.

Methods: Under IRB approval at Sam Houston State University, 22 college-aged participants were recruited to self-report alcohol use and provide biological samples twice weekly over 8 weeks of study (16 collections). Weekly surveys captured detailed alcohol consumption, including beverage type, quantity, and drinking frequency. At each time point, oral fluid, urine, and venous blood were collected and stored under refrigeration until analysis.

Whole blood (75 µL) collected in lavender-top tubes were protein precipitated with 0.5 mL 50:50 acetonitrile: isopropanol containing isotopically labeled internal standards. Following centrifugation, the supernatant was filtered, diluted, and analyzed using a validated LC-MS/MS method for POPEth and PLPEth (10–400 ng/mL). Oral fluid samples collected in Quantisal® devices were processed via solid-phase extraction and quantified using a validated LC-MS/MS method for EtS (12.5–2500 ng/mL). Urine samples collected in specimen cups (without preservatives) were initially screened using an EtG immunoassay (cutoff 500 ng/mL) and subsequently confirmed by a validated LC-MS/MS method for EtG (500-10,000 ng/mL) and EtS (100-10,000 ng/mL).

Results: Survey data was compared to analytical results for POPEth, PLPEth, EtG, and EtS. Drinking behaviors could be classified into low-level/intermittent drinking, episodic/clustered drinking, and moderate-to-high/frequent drinking. Blood PEth concentrations ranged from 10.6-198 ng/mL for POPEth and 10.5-259 ng/mL for PLPEth. No EtS was detected in oral fluid, and urinary EtG and EtS showed variable positivity. In cases where 1–2 drink events with irregular frequency were reported, EtG/EtS were rarely positive, while POPEth/PLPEth levels were either undetected or below 20 ng/mL. Periods of abstinence interspersed with moderate

drinking episodes produced intermittent positivity for EtG/EtS, while POPEth/PLPEth increased after drinking events and declined during gaps. In repeated moderate-to-high intake with multiple events, EtG/EtS were often negative between events, while POPEth/PLPEth remained consistently elevated with gradual decreases during brief reductions in drinking.

Discussion: Light and sporadic alcohol consumption generally produced no detectable PEth concentrations; however, sustained or repeated alcohol intake resulted in markedly increased PEth concentrations. Notably, PEth remained detectable in some cases where EtS and EtG were no longer present, highlighting its extended detection window and utility for identifying repeated or cumulative alcohol exposure beyond the timeframe of traditional direct metabolites.

Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

O-154

Targeted Toxicology: Establishing Overdose Biosurveillance in Vermont with a 75-Compound Panel

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Abstract

Introduction: In 2024, Vermont recorded 207 confirmed overdose-related deaths, ranking 9th per capita among states most impacted by the overdose crisis despite its small population. To improve situational awareness of the evolving drug supply, the Vermont Department of Health Laboratory (VDHL), supported by the CDC's Overdose Data to Action (OD2A) program, established a biosurveillance initiative to characterize substances detected in patients presenting to emergency departments with non-fatal overdoses. By integrating toxicological and demographic data, this first of its kind program in Vermont supports overdose prevention, risk reduction, and strategic public health response efforts.

Methods: De-identified non-fatal overdose specimens were analyzed at VDHL using an Agilent 6470 Triple Quadrupole with positive electrospray ionization coupled to an Agilent 1290 LC System. Urine specimens were analyzed using a "dilute-and-shoot" approach without pre-preparation. Isotopically labeled internal standards were added to support quantitation. Samples were analyzed using a targeted dynamic multiple reaction monitoring (dMRM) assay capable of detecting 75 compounds through compound-specific ionic transitions and retention time windows.

Results: To reduce re-identification risk, demographic data were limited to statewide aggregation of sex at birth, age, and race as "white" or "BIPOC" (Black, Indigenous, and other People of Color). Specimens collected from October 2024 - November 2025 (n=94) were analyzed using a 44-compound assay. Specimens collected from December 2025 - February 2026 (n=41) were analyzed using a 75-compound method. Reported findings reflect urine testing only; serum testing was implemented in February 2026 and will be incorporated prior to conference presentation.

The mean age of nonfatal overdose patients was 40.8 years. More than 22% were within the ≤18 years and ≥65 years age groups combined. Vermont's general population is approximately 91% white and 9% BIPOC; among overdose patients, 78% were white and 17% were BIPOC. Race was unreported in 5% of cases.

Cocaine and metabolites (benzoylecgonine/cocaethylene) were detected in 67% of samples, followed by fentanyl/fentanyl analogs (47%), naloxone (42%), methamphetamine/amphetamine (33%), methadone/EDDP (31%), xylazine (30%), morphine-related compounds including 6-acetylmorphine (25%), and gabapentin (19%). After correction for parent drug/metabolite combinations, the mean number of detected drugs per sample was 4.1, with a maximum of 13 substances identified in a single sample.

Common polydrug combinations included cocaine + fentanyl (52%), cocaine + methamphetamine (38%), cocaine + methadone (35%), fentanyl + xylazine (35%), cocaine +

xylazine (35%), and cocaine + fentanyl + methamphetamine (33%). High-impact detections included 4-ANPP, bromazolam, carfentanil, and medetomidine. Twenty-three percent of samples did not contain any of the 75 targeted analytes.

Discussion: These data exhibit increasing complexity within Vermont's overdose landscape, characterized by extensive polysubstance use, emerging sedatives, and ultra-potent opioids. BIPOC individuals represented a disproportionately high percentage of overdose cases relative to the state population, while both youth and older adults accounted for substantial proportions of non-fatal overdoses, highlighting the need for targeted prevention strategies. Detection of compounds including 4-ANPP, carfentanil, bromazolam, medetomidine, and xylazine reflects an evolving drug supply with increased overdose risk and potential resistance to standard naloxone reversal. Although targeted mass spectrometry effectively identified known substances and trends, 23% of samples were negative for targeted analytes, underscoring need for investment in high-resolution, non-targeted mass spectrometry to improve detection of emerging compounds and strengthen overdose biosurveillance efforts.

Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

O-155

Comprehensive Drug Testing in Dried Blood Spots for Multi-Year Surveillance of Unregulated Drug Use in Rhode Island

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Abstract

Introduction: Dried blood spots (DBS) are an emerging patient sampling technique, allowing detection of compounds in blood years after collection with small sample volumes. As part of a randomized controlled trial in Rhode Island, blood spots were collected from participants who reported recent drug use from 2021 to 2023. These DBS, comprised of only 50-80 µL of blood before drying, were screened against a panel of 350+ prescription and over-the-counter drugs, unregulated substances, adulterants, and metabolites.

Objectives: This study aimed to validate a high-resolution mass spectrometry-based comprehensive drug test in DBS following the Official International Association for Therapeutic Drug Monitoring and Clinical Toxicology (IATDMCT) Guidelines for Development and Validation of Dried Blood Spot-Based Methods for Therapeutic Drug Monitoring. Additionally, we aimed to use this method on DBS samples from clinical trial participants who reported recent drug use in order to characterize drug use patterns and assess the presence of potent adulterants in the drug supply between 2021 and 2023.

Methods: DBS were collected by trained, non-medical personnel researchers, using Whatman DBS collection cards. The cards we allowd to dry overnight, then stored at -80°C until processing. Samples were then thawed, and subjected to a two-step extraction, aquoeous, then organic solvent-based, prior to injection into a high-resolution mass spectrometer (LC-QTOF-MS) for analysis. While the extraction protocol was optimized for the recovery of fentanyl and the drug supply adulterant xylazine, matrix effects (ME) and limits of detection (LOD) were determined for all 350 analytes of interest.The untargeted analysis method was qualitative, using drug identification criteria previously established in clinically-validated urine and serum-based methods.

Results: The LODs and MEs achieved would allow for DBS-based surveillance and monitoring, as they were compatible with concentrations relevant to the therapeutic ranges of all prescription drugs and metabolites described. For unregulated substances and adulterants, these parameters were comparable to those previously established in serum. While a direct DBS to whole blood sample comparison in the study population was not performed, LODs were equivalent to those in the clinically validated serum-based method, conferring comparable feasibility of exposure detections in surveillance efforts.

Of the 420 total samples, fentanyl and related analogs were detected in 102 (24.3%). The veterinary sedative xylazine, a potent adulterant recently designated as a national public health concern, was found in 32 samples (30.4% of fentanyl positive samples, 7.6% of overall samples). Other analytes of interest included cocaine (74.5%), methamphetamine (14.3%), and heroin (1.2%).

Discussion: Our comprehensive drug screen method by LC-QTOF-MS in DBS enables qualitative, definitive testing in traditionally vulnerable populations, who may not present to a healthcare center for routine testing. This provides insight into drug use patterns and drug supply variation otherwise inaccessible, valuable especially when triangulating with data from hospitals and drug checking information. Given its compatibility with easy, accessible sample collection, this study provides support for future use of DBS in clinical studies involving samples collected over long periods of time, allowing significant cuts on resources needed for transportation and storage, or with patients for which typical blood draws are not feasible, such as neonates or individuals with no access to healthcare.

Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

O-156

LC-MS/MS QTOF Analysis of River Water Identifies Contaminants of Environmental Concern by Non-Targeted Profiling

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Abstract

Introduction: Water-based epidemiological studies have yielded valuable insights into wastewater treatment processes and have enabled the identification of contaminants of environmental concern (CEC) in recent years. Both targeted and non-targeted analysis using full-scan mass spectrometry with data-independent acquisition (DIA-MS/MS) have been applied to detect emerging contaminants that may originate from treated or untreated wastewater as a result of storm overflows.

Objectives: In this study, river water samples were measured from both tidal and freshwater locations. Novel non-targeted analysis (NTA) profiling software was applied to identify key differences between and within sample groups.

Methods: River water samples were taken from 12 sites on the River Thames and 10 sites on the River Hogsmill. Samples of 500 µL were prepared using Millipore centrifugal filters (Ultrafree - MC – GV, Dispose - PVDF 0.22um) for 4 min at 12,000 g. Aliquots from each sample were taken to create a pooled QC to run throughout the analysis. Samples were measured by direct injection (40 µL) and separated using a generic reversed phase forensic toxicology gradient using a Shim-pack Biphenyl column (100x2.1mm 2.7 µm). Analysis was performed by ESI MS-DIA MS/MS (LCMS-9050, Shimadzu Corporation) m/z 100-1000, in positive and negative ion modes. Data was processed using Insight Profiler software which performed feature detection, data alignment and compound identification.

Water samples were collected from multiple sites on two rivers to assess whether non-target analysis could differentiate samples based on location. The Hogsmill River is an urban freshwater tributary, while the River Thames is tidal. Aliquots from each sample were combined to produce a pooled quality control (QC) sample. System conditioning injections of the pooled QC were performed prior to analysis, followed by a randomized sample run with QC injections every four samples. Samples were measured using HRMS LC-DIA-MS/MS with electrospray ionisation (ESI) in positive ion mode, followed by a second analytical batch in negative ion mode. The Insight Profiler method was configured to detect trace level components in all samples, align detected features, filter features based on data quality criteria and identify each component with high confidence using multiple MS/MS data repositories.

Results: Samples taken downstream of the Hogsmill River facility contained a high number of pharmaceutical compounds and metabolites, including venlafaxine, desmethylvenlafaxine, tramadol, O-desmethyltramadol, gabapentin, ketamine, clarithromycin, metronidazole, carbamazepine, and lidocaine, whilst upstream samples showed a markedly lower distribution and concentration of pharmaceutical compounds providing evidence of the impact of the wastewater treatment facility (WWTF) on downstream profiles. It was also notable that acetaminophen (paracetamol), recognized as a well-established tracer of wastewater influent, was detected at all sampling sites at relatively consistent but low levels.

Discussion: The disparity in concentration and number of pharmaceutical compounds found upstream versus downstream of the WWTF, identified with Insight Profiler data processing, highlights the need for systematic monitoring of river samples close to WWTFs.

Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

O-157

Wastewater-Based Epidemiology of Prescription Opioids in Reykjavík, Iceland Between 2024–2025

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Abstract

Introduction: Wastewater-based epidemiology (WBE) is a complementary approach for monitoring drug consumption at the population level. By measuring drug residues in municipal wastewater, WBE provides near-real-time information on community drug use, complementing clinical data and prescription records. In Iceland, opioid prescribing has received increased attention in recent years due to concerns about misuse and efforts to promote more cautious prescribing practices. In addition, some prescription opioids are imported illegally, meaning their consumption would not be captured in prescription databases. WBE therefore offers a valuable tool for monitoring both prescribed and non-prescribed opioid use in Iceland.

Objectives: This study aimed to quantify the most common prescription opioids in municipal wastewater from Reykjavík and compare wastewater loads with prescription data to evaluate temporal trends.

Methods: Composite wastewater samples were collected over a 7-day period once per month between January 2024 and December 2025 from the Klettagarðar wastewater treatment plant in Reykjavík, Iceland. Samples were extracted using Oasis HLB solid-phase extraction cartridges, resulting in a 250-fold concentration. Oxycodone, tramadol, morphine, codeine, fentanyl, and norfentanyl were analyzed using a validated UPLC–MS/MS method. Wastewater loads were normalized to mg/day/1000 inhabitants and compared with dispensed defined daily doses (DDD) for the same opioids.

Results: Mean annual wastewater loads decreased from 2024 to 2025 for morphine, codeine, oxycodone, and tramadol. Fentanyl and norfentanyl concentrations were below the detection limit in all tested wastewater samples. The mean load (mg/day/1000 inhabitants) declined significantly for morphine (-34.0%, $p<0.05$) and tramadol (-27.3%, $p<0.05$), while non-significant reductions were observed for oxycodone (-21.6%) and codeine (-17.1%). Prescription data showed downward trends in dispensed DDD over the study period for morphine, codeine, oxycodone, and tramadol, while fentanyl prescriptions remained stable. Moderate to strong positive correlations between monthly wastewater loads and prescription data were observed for morphine ($r = 0.50$, $p < 0.05$), oxycodone ($r = 0.45$, $p < 0.05$), and tramadol ($r=0.64$, $p < 0.001$). No significant association was observed for codeine ($r=0.26$, $p=0.25$).

Discussion: Wastewater analysis showed declining opioid loads in Reykjavík between 2024 and 2025, consistent with reductions in prescription data. The weaker agreement for oxycodone may reflect contributions from non-prescribed sources, while the lack of association for codeine likely reflects greater variability between wastewater measurements and dispensed prescription data. Notably, neither fentanyl nor norfentanyl was detected in wastewater despite ongoing prescription use averaging approximately 347 DDD/month. This

likely reflects the combination of low mass consumption associated with the high potency of fentanyl and the extensive metabolism of the drug, resulting in very low concentrations of parent fentanyl entering wastewater. Unlike many North American wastewater studies where fentanyl and its analogues are routinely detected, the opioid profile in Reykjavík was dominated by oxycodone, tramadol, morphine, and codeine. These findings support wastewater-based epidemiology as a complementary tool for monitoring pharmaceutical opioid use and evaluating public health interventions.

Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

O-158

Toxicological Profiling of Liberian Local Remedies: Implications for Health and Safety

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Abstract

Introduction: Traditional medicines remain a cornerstone of healthcare across sub-Saharan Africa, where up to 80% of the population relies on herbal and locally prepared remedies for primary care. These preparations hold deep cultural significance, serving as intergenerational treatments for pain, infections, reproductive health, and neonatal care. However, the safety of these products is often assumed rather than verified. Previous literature demonstrates contamination, adulteration, and variability in composition of local remedies.

Objectives: The primary objective of this study was to evaluate twenty-five Liberian local remedies for the presence of pharmacologically active drugs of abuse (DOA) using multiple analytical platforms. A second objective was to contextualize toxicological findings within the broader literature on herbal remedy safety, cultural reliance, and the challenges of analytical surveillance in low-resource environments.

Methods: Twenty-five samples were acquired from herbalists and retail outlets. Solid samples were homogenized in three solvents (H_2O, 50:50 MeOH:H_2O, and <5% ACN), while liquid samples were analyzed directly. Analysis included Randox ELISA for broad DOA detection, MedTox ELISA for narrow-DOA liquid screening, liquid chromatography-tandem mass spectrometry (LC-MS/MS) for high-specificity qualitative confirmation, headspace gas chromatography (HS-GC) for volatile identification and quantification, and gas chromatography-mass spectrometry (GC-MS) for constituent identification against national databases. Assays were repeated to evaluate reproducibility and investigate inconsistencies.

Results: Randox ELISA identified seven of twenty-five samples as positive for stimulant, opioid, or benzodiazepine-class immunoassay signals. MedTox ELISA results were largely negative except for one liquid remedy which showed inconsistent detection of amphetamines and benzodiazepines across dilutions. LC-MS/MS repeatedly confirmed xylazine in an “everlasting leaf” sample obtained through an authorized intermediary, while a sample harvested directly from its native soil consistently tested negative. HS-GC analysis revealed elevated ethanol content in several samples, including products labeled “nonalcoholic.”

Discussion: The identification of stimulant, opioid, benzodiazepine-class signals, and xylazine in traditional remedies aligns with broader global literature documenting contamination in herbal medicines. Xylazine’s presence in an “everlasting leaf” herbal supplement mirrors its emergence in illicit drug supplies with discrepancies between samples, which suggests supply-chain contamination rather than intrinsic plant chemistry. While the source of this contamination is unknown, these findings are consistent with phenomena noted in WHO reports on quality lapses in unregulated herbal markets. The detection of xylazine is particularly concerning given reports of “everlasting leaf” being used for neonatal umbilical stump care with associated perinatal deaths. While these assays suggest possible DOA, results should be interpreted cautiously due

to potential limitations inherent to the analytical techniques, such as potential cross-reactivity to structurally similar non-DOA constituents.

Nonetheless, these findings carry meaningful public health implications in low resource settings where traditional medicines are culturally important and often the first line of care.

Conclusion: This multi-assay evaluation identified stimulant, opioid, and benzodiazepine-class immunoassay signals, confirmed xylazine contamination, and documented clinically relevant alcohol levels in several widely used Liberian remedies. These findings underscore the need for strengthened regulatory oversight and culturally grounded public health messaging to ensure the safety and integrity of traditional medicines in low-resource settings.

Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

O-159

Driving Impairment and TDM Adjustment Pre and Post Liver Transplant

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Abstract

Introduction: Symptoms of organ failure in an individual may present as possible drug impairment in clinical and forensic settings. Patients may exhibit signs of fatigue, confusion, jaundice, low urine output and shortness of breath, which may be mistaken for drug effects. In addition, liver dysfunction alters the metabolism of drugs, requiring dose adjustment. It is important that law enforcement understand the presentation of individuals with possible medical emergencies so that the response is proportional.

Objectives: The aim of this study is to present the case history of a 62-year-old male diagnosed with cirrhosis of the liver, in which clinical signs and symptoms of liver dysfunction resulted in a traffic stop with suspicion of driving while under the influence of alcohol/drugs. In addition, following organ transplantation, we present unusual therapeutic drug monitoring [TDM] findings of the immunosuppressant, tacrolimus, emphasizing the importance of establishing a complete clinical profile to ensure a positive outcome.

Methods: In November 2024, the male was driving home from work at approximately 9 pm and was stopped by local law enforcement due to driving 20 mph under the speed limit. The officer did not detect the odor of alcohol, or red eyes and upon questioning, the subject appeared to be confused, lacked awareness of surroundings, and exhibited slurred speech. The male was transferred by the local fire department to a medical facility where he was diagnosed with hepatic encephalopathy caused by hyperammonemia. A blood ammonia level of 168 (normal range 16-60 umol/L) was determined using the Roche Cobas 6000 by enzymatic, ultra-violet kinetic methodology (Roche NH3L2, Ammonia II test) by reacting ammonia with a-ketoglutarate and NADPH in the presence of glutamate dehydrogenase.

Results: Due to the medical findings following this incident the patient was put on the transplant list and in March 2025 received an organ from a deceased donor. The immunosuppressant, tacrolimus, was added to the medication regime one day later. Daily in-house testing by chemiluminescent immunoassay using the Abbott Alinity i, (normal range 5-20 ng/mL) was conducted. The blood concentration was consistently in the 3.2-4.4 ng/mL range. The dosing regimen was changed to sublingual 7 mg BID. Thereafter, the patient was able to maintain a steady state concentration in the therapeutic range between 5-10 ng/mL.

Discussion: Unexpected causes of impaired driving due to medical conditions will be encountered by law enforcement. Rapid identification and appropriate response to these situations is critical and should be included in officer training.

Patients should undergo therapeutic drug monitoring during surgical recovery since this may reveal genetic variations. Fast tacrolimus metabolism in liver transplant recipients is defined by a low trough concentration/daily dose ratio, typically <1.09 ng/mL per mg, indicating rapid tacrolimus metabolism due to CYP3A5*1 genetic polymorphism. In this case, early ratios were less than 0.50. Dose alterations while in the safety of a hospital setting allows rapid response to TDM and other treatment failures.

Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

Conflict of Interest

The first author is the subject of the case report (HIPAA compliant).

O-160

2026 Update on Standards Development Activities in Forensic Toxicology

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Abstract

Introduction: The 2009 National Research Council (NRC) report, *Strengthening Forensic Science: A Path Forward*, critically reviewed the forensic sciences in the United States, and encouraged the development and implementation of standards.[i] In response to the NRC report, the National Institute of Standards and Technology (NIST) created the Organization of Scientific Area Committees (OSAC) for forensic science, with the goal of "Strengthening the nation's use of forensic science by facilitating the development of technically sound standards and guidelines and encouraging their use throughout the forensic science community."[ii] The various OSAC Subcommittees draft discipline specific documents. Their seed documents are then forwarded to a standards developing organization (SDO) to be developed and published through consensus-based processes. They also review published standards for inclusion on the OSAC Registry which serves as a central repository of standards considered to be high-quality and technically sound.

The American Academy of Forensic Sciences (AAFS) is a multidisciplinary professional organization that provides leadership to advance science and its application to the legal system. Following the NRC report and the creation of OSAC, AAFS recognized the unique and important role it should have in forensic science standards. AAFS created the Academy Standards Board (ASB) as the first US SDO dedicated entirely to the development and maintenance of forensic science standards. ASB is accredited by the American National Standards Institute (ANSI). Its procedures provide for due process based on openness, balance, and consensus; and they ensure that all interested and affected parties have an opportunity to participate. The American National Standards published by ASB are available at no charge to the general public.[iii]

Objectives: This presentation will provide attendees with updates on the standards development activities in forensic toxicology, including ASB published American National Standards, ASB toxicology standards on the OSAC Registry, and documents in the draft stage at both ASB and OSAC. Tools and resources available to assist with implementation will also be discussed.

Discussion: Forensic standards impact all aspects of daily work in forensic toxicology, including initial method validation, daily operations, staff training and competency, and reporting and testimony. Many forensic science service providers are voluntarily incorporating standards into their practices, and the courts are recognizing the benefit these standards have toward establishing consistency and reliability.

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[ii] https://www.nist.gov/osac

[iii] https://www.aafs.org/academy-standards-board

Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

O-161

A Novel Model to Discern Exogenous Gamma-Hydroxybutyric Acid (GHB) in Postmortem Cases

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Abstract

Introduction: Gamma-hydroxybutyric acid, GHB, occurs in the human body endogenously and is used medicinally and recreationally as an exogenous substance. Interpretation in postmortem casework is further challenged by its increase following death and decomposition. Over the years, different thresholds to discern endogenous from exogenous GHB in postmortem blood have been suggested, 50 and 30 mg/L.

Objectives: The aim of this study was to develop and validate a novel model to classify postmortem casework as acute exogenous GHB use (AEU), possible exogenous use (PEU), or no indication of GHB use. This presentation provides a user-friendly HTML tool along with updated applicability validation studies following previously shared conceptual work.

Methods: Since 2020, the Office of the Chief Medical Examiner for the City and County of San Francisco, California, has routinely analyzed all postmortem casework for GHB and 200 other drugs and metabolites by LC-MS/MS. The GHB LOD for blood and urine were 5 and 10 mg/L, respectively. The model development dataset was comprised of postmortem cases between 2020 and 2023 with paired peripheral blood and urine concentrations (n = 552). Cases were classified as acute exogenous GHB use (AEU), confirmed exogenous use (confirmed PEU), or no indication of GHB use, and plotted as a hybrid of existing guidelines with urine-to-blood ratios (y-axis) versus blood concentrations (x-axis). Boundaries between these cohorts were visually identified and simple first-order functions derived to develop the model. Validation of the model was evaluated with the development dataset and further assessed using internal applicability data, positive GHB detections from San Francisco OCME between 2024 and 2025 (n = 33). Validation using an external applicability dataset was also studied with GHB data from 2017 to 2024 (n = 2233) obtained from colleagues from Laboratoire de sciences judiciaires et de médecine légale in Quebec, Canada.

Results: The existing recommendations for PEU were generally corroborated and the introduction of a model to discern AEU increases the utility of paired peripheral blood and urine concentrations in postmortem GHB. The proposed guideline is intentionally liberal in suggesting PEU to warrant review and corroboration of death scene artifacts of reported history and thus, layers a minimum peripheral blood concentration of 5 mg/L. In contrast, the proposed guideline for suggesting AEU is deliberately conservative and increases the minimum peripheral blood concentration significantly to 50 mg/L. Both guidelines maintain the relevance of urine concentrations by incorporation of the urine-to-blood ratios.

Discussion: A novel model is presented to assist in the interpretation of postmortem GHB when peripheral blood and urine concentrations are available, whether suggesting acute endogenous use or possible endogenous use. We recommend universal analysis of blood for GHB, made possible by comprehensive multi-drug class analytical methodology, and when detected above 50 mg/L, additional GHB analysis of urine. The inclusion of GHB in routine, systematic toxicology analysis approaches, enabled significant collection of GHB concentrations and thus the development of improved guidelines and provision of a user-friendly HTML tool to interpret postmortem GHB concentrations.

Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

O-162

GHB in Québec (Canada): The Data and the Story

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Abstract

Introduction: As previously published in a 5-year study of drivers under the influence of drugs (DUID) (Vaillancourt et al. *Accident Analysis & Prevention* (2021) 149:105832), gamma-hydroxybutyrate (GHB) is frequently used for recreational purposes in the province of Québec (Canada). Because of its endogenous nature, it can be difficult to distinguish a "normal level" from exogenous intake. Various positivity thresholds for exogenous use are proposed in the literature for blood and urine. Since 2017, the Laboratoire de sciences judiciaires et de médecine légale (LSJML) in Québec systematically analyzes GHB in both blood and urine for postmortem and antemortem cases. A large amount of data to study antemortem GHB concentrations found in DUID and drug facilitated crimes (DFC) cases is therefore available.

Objectives: To present a large dataset of GHB antemortem concentrations in both blood and urine, with descriptive statistics including the mean, median, and percentiles; to assess the prevalence of GHB in Québec between 2017 and 2024 in antemortem cases.

Methods: Antemortem cases submitted to LSJML between 2017 and 2024 were reviewed for confirmed identification of GHB results in blood and urine. GHB was quantified in blood by LC-MS/MS between 3 and 300 mg/L, while urine concentrations were estimated using a one-point (9 mg/L) calibration curve (Desharnais et al. (2025) TIAFT 2025, Auckland). Prevalence of GHB was evaluated with positivity thresholds of 5 mg/L in blood and 10 mg/L in urine as recommended by the Canadian Criminal Code (RSC 1985, c C-46) and ANSI/ASB Standard 121.

Results: From 2017 to 2024, a total of 15 580 DFC and DUID cases were analyzed for GHB, with blood and/or urine samples. 97% of blood samples analyzed were <3 mg/L. For the remaining samples, concentrations ranged from 3.1 to 487 mg/L (median=102, mean=107, 5th percentile=4.7, 95th percentile=250; n=472). GHB was detected in 25% of urine samples, with estimated concentrations between 0.10 and 14 335 mg/L (median=1.4, mean=424, 5th percentile=0.47, 95th percentile=2 520; n=3 955). Prevalence of GHB was evaluated based on recommended positivity thresholds. Less than 1% of samples were positive in DFC cases (n=4 314), whereas 13% of DUID cases (n=11 266) were positive.

Discussion: The positivity rate, calculated according to the recommended thresholds for antemortem cases, suggests a high prevalence of recreational use in the province, especially for DUID. It is important to acknowledge that, although supported by literature, the thresholds remain somewhat arbitrary and may lead to under- or overestimation in prevalence estimates.

Distinguishing exogenous use from endogenous GHB presence is central to DUID and DFC cases. Yet, the extensive dataset of GHB concentrations from both blood and urine samples presented in this study does not show two distinct populations leading to the identification of such a differentiating threshold. Unfortunately, the algorithm developed by Pearring et al.

(*Journal of Analytical Toxicology*, (2026) In Press.) for postmortem cases is not applicable in antemortem cases, since paired blood and urine samples, collected at the same time, are rarely available. A different approach will thus be required to probe antemortem exogenous consumption.

Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

O-163

Streamlined Approaches to Measurement Uncertainty Evaluation in a 200+ Drug Analysis

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Abstract

Introduction: Measurement uncertainty (MU) is an estimate of the potential variability of a quantitative measurement and is obtained through evaluation of the measurement process and the materials and equipment used in such a process. Measurement uncertainty is a critical component of forensic toxicology, increasing confidence in the performance of a method. However, the evaluation of MU for methods encompassing over 200 drugs can be labor-intensive and prone to errors when performed manually. Streamlining MU evaluation, at a large scale, is therefore essential to maintain efficiency without compromising quality.

Objectives: This project aimed to develop a streamlined and efficient approach for evaluating MU in a 200+ drug analysis. A key objective was to create a workbook template utilizing Microsoft Excel, integrating LabLink, a quality assurance software, to semi-automate systematic data processing and minimize human error (e.g., transcription error) while still following the Academy Standards Board (ASB) 056 and the National Institute of Standards and Technology (NIST) 8-step process.

Methods: An Excel workbook was created to combine data sources, perform calculations, and report MU values into a single template. Sources of uncertainty were identified, including contributions from calibration standards, calibrated labware, and any potential bias. Data from routine quality control samples, housed in LabLink, were imported into the workbook template. Built-in formulas and Excel Power Query were used to semi-automatically calculate standard uncertainties, combine standard uncertainties, and expand uncertainty using the appropriate coverage factor. When feasible and reasonable, a conservative approach was followed throughout each step of the evaluation. When a selection between observed variability and maximum allowable variability existed, maximum allowable variability was utilized.

Results: The Excel workbook template significantly reduces the time required for MU evaluation, especially for our drug analysis that targets 226 analytes. Semi-automated calculations eliminated repetitive manual steps and improved consistency in applying formulas. Evaluating MU for a method can be laborious, but using a standardized workbook template streamlines the process and can reduce the time required to just a single day. By utilizing the maximum allowable variability for each component, it minimized changes needed during ongoing re-evaluation of MU. Additionally, during re-evaluation, with the use of LabLink, the amount of time and effort to refresh and use new routine quality control data to calculate reproducibility is significantly reduced. Over the course of four years (2021–2025), four re-evaluations were conducted, each occurring approximately 7–12 months apart. Whether the addition of new components or updating quality control data for one or all components, the amount of active time to re-evaluate has taken no more than an hour.

Discussion: The implementation of an Excel workbook template for MU evaluation offers a practical solution for high-throughput forensic toxicology laboratories. While the Excel workbook template is accessible and adaptable, validations by peers are essential to ensure reliability and intended function and calculations. This streamlined approach is particularly advantageous for large multi-analyte panels, can be readily applied to other types of analytical methods, and can be easily re-evaluate when needed. Future work may explore integration with laboratory information management systems (LIMS) or to fully automate the process to further enhance efficiency and data integrity.

Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

O-164

Mathematics-Based Best Practices and Software Tools for the Method of Standard Addition

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Abstract

Introduction: Interest in the method of standard addition (MSA) has seen an increase in popularity recently. However, there is a glaring lack of information in scientific literature and laboratory standards guidance regarding best practices. Furthermore, little work has been done to verify the validity of applying quadratic and/or weighted calibration models to MSA, or the calculation of measurement uncertainty (MU).

Objectives: Define and validate mathematics-based best practices for MSA quantification, including selection of upspikes, weighting, and calibration model order as well as calculation of MU. Additionally, develop and validate software tools to perform these calculations and generate reports.

Methods: Simulated data sets were generated from experimental response curves of multiple analytes across different LC-MS/MS instrumentation, and an actual experimental data set for benzodiazepines was generated. Precision and accuracy of the simulated data sets were used to select the most accurate mathematical approach. R® and RStudio®, and VBA® for Microsoft® Excel® were used to create the calculation tools presented. The experimental data was used for model and software development and verification.

Results: Expected results were compared to EZMSA R and EZMSA Excel results to validate calculation accuracy and consistency.

Discussion: Best MSA practices begin with selection of correct upspike concentrations. The highest upspike should be as high as possible without detector saturation, generally more than five times the estimated unknown concentration while the rest of the upspikes should be clustered at the low end of the curve since that is where variance is lowest. If possible, the number of replicates at each level should be increased rather than increasing the number of levels. To determine whether a quadratic or linear regression is appropriate, the confidence interval of the b_2 parameter (y-intercept) of the regression equation can be evaluated. To simplify MU calculations, a flipped coordinates technique can be used in which the absolute value of the y-intercept is the concentration of the unknown analyte, and the measurement uncertainty is simply the confidence interval at that concentration. Due to the mathematical complexity of calculating and applying the correct weighting to a quadratic model, a coordinate-flipped model, or both, the EZMSA R and EZMSA Excel tools were developed. Both use the most statistically significant model by default but can be forced to a linear or quadratic model, and 1/variance, $1/x^2$, 1/x, or 1 weighting. These tools have been validated using simulated and experimental data.

Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

O-165

Application of Risk Management to Analytical Method Validations

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Abstract

Introduction: Applying risk management principles to analytical test methods during the development phase is the best practice to ensure robust assay performance and reliable results. Early identification of high-risk process parameters enables the application of effective mitigation and control strategies to ensure assays consistently meet their intended use. This abstract describes Abbott Toxicology's approach to risk management during analytical test method development and validation to ensure reliable results in forensic and clinical testing-as-a-service.

Objectives: This presentation will detail the implementation of the risk management framework at Abbott Redwood Toxicology Laboratory, including the design and application of a risk registry, risk evaluation methodology, and risk mitigation activities aligned with regulatory expectations, ICH Q9 (R1) quality risk management principles, and CLSI EP18 guidance for laboratory testing processes. The objective is to present how early application of risk management informs user requirements, including their potential effects on failures, likelihood of occurrence, and risk criticality to prevent costly errors.

Methods: A standard operating procedure for test method development is employed to systematically assess potential failure modes in analytical methods and instruments using Process Failure Mode and Effects Analysis (PFMEA). Risks are identified across the pre-analytical, analytical, and post-analytical phases of testing. Each risk of failure is scored based on Severity, Occurrence, and existing Mitigations or Controls. These scores are multiplied to calculate an overall Risk Priority Number (RPN), which categorizes the risk as low, medium or high and prioritizes the mitigation activities. Risk assessments are conducted during the method development phase and mitigated via test method validation.

Results: The risk management process enables proactive identification and prioritization of data integrity, analytical performance, and result interpretation risks relevant to method validation. Use of this risk-based approach supports the targeted mitigation of higher-priority risks and informed validation design decisions as represented by the following:

Data integrity risks are addressed through the implementation of system controls. For example, enabling audit trails, using unique user credentials on instruments, and restricting unauthorized system access.

Analytical result inaccuracy risks, such as control material excursions and instrument performance variability, are managed through uncertainty calculations, the use of statistical process control charts (SPC), routine monitoring of analytical result trends, and adherence to preventive maintenance schedules.

Risks related to specimen result reversal decisions are evaluated by considering analyte stability in their respective biological matrices, storage conditions, sample age, and sample processing workflows. Mitigation strategies include defining acceptance criteria, establishing

storage and handling controls, and characterizing sample stability parameters for technical bulletins to stakeholders.

Discussion: Human factors contributing to risk across the pre-analytical, analytical, and post-analytical phases are evaluated at each process step. Discussion of the identified potential failure modes, their rankings by severity and likelihood, and the actions taken to implement preventive and detective controls. Practical examples will include risk scoring, mitigation strategies, and continuous risk review and will be presented to demonstrate effective integration of risk management principles into analytical method validation.

Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

O-166

Multi-Factor Fermentation Experiment in Ante-Mortem Blood Alcohol Testing

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Abstract

Introduction: A frequent argument in impaired driving cases involving blood alcohol concentration (BAC) is that ethanol may have been artificially produced post collection via fermentation. Previous studies have addressed individual factors such as blood tube expiration, collection method, and storage conditions, and blood glucose level.

Objectives: This study aims to combine the previously mentioned factors into a single study, specifically within 10 mL BD Vacutainer® Gray Top Tubes.

Methods: We compared expired and unexpired blood collection tubes and exposed them to a variety of environmental conditions intended to simulate various collection scenarios. These scenarios consisted of mimicking recommended collection techniques, as well as allowing the tubes to sit uncapped for one hour on a laboratory bench, in a restroom, or outdoors on an uncovered smoker's patio. These scenarios intentionally broke sterility of tubes for an extended period of time. Five milliliters of human bank blood was added to each tube via a sterile syringe. Blood glucose levels were tested and found to be >300 mg/dL. Half of the samples were artificially spiked with ethanol while the others remained ethanol negative. After preparation, all samples were tested for their baseline BAC. Blood tubes were further subjected to three storage conditions: refrigerated, room temperature, and in a vehicle trunk and had the BAC tested again after a two-week storage period. Following all testing in July 2025, all samples were stored in a refrigerator for six and a half months then retested in January 2026 to simulate outsource testing that may be court ordered at the request of an attorney.

Results: The results of this study again illustrate that there is no adverse effect on BAC levels when using a Vacutainer® past its expiration. Samples that were baseline negative for alcohol remained alcohol negative regardless of sterility or storage conditions. No statistically relevant differences in spiked BAC levels were observed among the simulated collection environments. The greatest decrease in ethanol concentration between the initial test and two-week environmental exposure occurred in samples stored in the vehicle trunk, followed by those stored at room temperature, with refrigerated samples showing the smallest decrease. All samples were retested after six and a half months of refrigerated storage and showed further decrease in ethanol levels across all positive samples regardless of expiration or sterility status. Furthermore, no sample that was originally alcohol negative had any amount of ethanol detected after storage.

Discussion: This study adds additional doubt that alcohol formation in ante-mortem samples collected in gray stopper tubes with sodium fluoride and potassium oxalate would occur, even in uncontrolled diabetic samples. No sample initially negative was found to later be positive under any conditions. Positive samples lost ethanol over the course of this study under all sterility, storage, and expired vs unexpired sample collection conditions.

Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

O-167

Δ8-THC in the Marketplace and the Laboratory: Δ8-THC Product Labeling Accuracy and Standard Urinary Drug Testing

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Abstract

Introduction: The rapid expansion of hemp-derived Δ8-tetrahydrocannabinol (Δ8-THC) products has outpaced both accurate labeling standards and drug testing protocols. Current urinary immunoassay (IA) screening was designed to detect Δ9-THC and may perform poorly for Δ8-THC.

Objectives: This study addressed two related gaps: (1) the accuracy of Δ8-THC content labeling of commercially available products, and (2) the performance of standard urinary drug testing following controlled Δ8-THC administration via oral and vaporized route. Together, these analyses will allow for assessment of what drug testing performance under ideal laboratory conditions implies for real-world cannabinoid detection among consumers using products with uncertain content composition.

Methods: 139 commercially available cannabinoid products were chemically analyzed to assess labeling accuracy with respect to Δ8-THC content. Products were categorized based on whether Δ8-THC was detected, labeled, and whether labeled amounts fell within an acceptable range of measured values (within +/-10%). Separately, urine samples obtained from healthy adults exposed to Δ8-THC in a controlled human laboratory study were tested using standard Δ9-THC-COOH immunoassay tests (20, 50, and 100 ng/mL cut-offs) and compared against GC/MS testing of both Δ8-THC and Δ9-THC (15 ng/mL cutoff) following single acute dose administration of oral (n=19; 10, 20, 40mg) and vaporized (n=20; 10, 20, 40mg) Δ8-THC.

Results: Product dose-labeling was highly inaccurate. 70 products listed Δ8-THC on the product label; 29 of which contained Δ8-THC but provided no specific quantity on the label from which to test accuracy. Among those with a labeled amount (range: 184-11844mg), under-labeling (n=14) was more common than over-labeling (n=10), or accurate labeling (n=5) to the amount measured, with 12 products not measured. Notably, Δ8-THC was detected in 39 products that made no mention of it on the label, meaning consumers may be unknowingly exposed to Δ8-THC. Following oral Δ8-THC, positive test results occurred in 77%, 72%, and 64% at the 20, 50, and 100 ng/mL cut-offs, respectively, suggesting a high level of cross-reactivity between Δ8-THC exposure and IA testing for detecting Δ9-THC use. GC/MS testing showed no detection of Δ9-THC-COOH.

Discussion: Our studies showed significant concerns with respect to product label accuracy for Δ8-THC. Many products contained higher than labeled doses, failed to provide dose information, or contained Δ8-THC in the absence of any mention on the product. In the controlled dosing laboratory study, there was a high level of cross-reactivity with Δ9-THC IA tests, which would result in a substantial number of false positives in standard drug testing screens. In real-world setting, where consumers are exposed to unknown and uncontrolled

quantities of Δ8-THC through mislabeled or unlabeled products, there is a need to establish a reliable standard of drug testing for confirming illicit use of Δ8-THC products in workplace test programs. There is also a need for validated Δ8-THC-specific product labeling and testing standards for the evolving cannabinoid marketplace.

Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

O-168

Lessons Learned the Hard Way: A Multi-Year Implementation Case Study in a Forensic Toxicology Laboratory

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Abstract

Introduction: In the scientific community, access to new instrumentation is highly valued; however, the implementation is not always a straightforward process. In 2020, the Metro Nashville Police Department Crime Laboratory Toxicology Section obtained funding for the acquisition of four instruments: two quadrupole time-of-flights (QTOF) and two liquid chromatograph tandem mass spectrometry systems (LC-MSMS). These upgrades were intended to improve analytical sensitivity and expand testing scopes. While implementation was anticipated to be relatively simple, the process proved significantly more complex. Instrument integration was influenced by staffing instability, administration delays, and infrastructure limitations. This case study outlines the challenges encountered and the lessons learned during a multi-year implementation process.

Objectives: Highlighting the administrative staffing, technical, and infrastructure challenges that can significantly impact the implementation and validation of instrument upgrades.

Methods: A retrospective evaluation of instrumentation implementation, method development activities, and laboratory operational challenges was conducted. This evaluation identified factors contributing to delays in bringing new instruments online.

Results: In September 2020, a quote was submitted for new instrumentation; however, final approval was not obtained until November 2023. During this period, the toxicology section experienced significant staffing challenges. The laboratory typically operated with three scientists and one supervisor, but by 2020 staffing had declined to two scientists and one supervisor. By summer 2021, both scientists had departed, leaving only the supervisor to manage operations. As a result, in-house casework was suspended and outsourced.

Instrument installation occurred in January 2024 following resolution of administrative and contractual delays. Despite a prior vendor site visit, installation revealed that existing autosamplers were incompatible with updated software, requiring procurement and installation of new autosamplers in summer 2024. Additional technical challenges occurred with the QTOF tuning solutions, which were eventually resolved, allowing infusion experiments and method development to begin in late 2024.

Concurrently the laboratory's water purification system and hydrogen generators failed, requiring temporary workarounds. In summer 2025, decreased instrument response was observed on both QTOF and LC–MS/MS systems during method development. Troubleshooting included evaluation of the nitrogen generator, installation of additional filters, and inspection of facility gas lines. Root cause analysis identified improper welding of gas lines, resulting in oily residue contamination which required the replacement of all gas lines. Gas line replacement was completed in spring of 2026, and method validation will resume in the summer of 2026.

Discussion: While new instrumentation is often viewed as a straightforward upgrade, this case demonstrates that implementation can become a complex, multi-year process. These findings emphasize the importance of comprehensive pre-installation planning, including verification of hardware/software compatibility with vendors, thorough infrastructure evaluation, and development of realistic timelines.

Failures in supporting systems, such as gas delivery and water purification, had direct and significant impacts on instrument performance, underscoring the dependence of advanced analytical platforms on laboratory infrastructure. Additionally, staffing continuity played a critical role in the ability to implement, troubleshoot, and advance method development.

Successful integration of new technologies requires more than acquisition of instrumentation; it depends on alignment between personnel, infrastructure, and administrative processes. Without this coordination, implementation may shift from an anticipated “plug-and-play” process to a prolonged “troubleshoot-and-adapt” effort.

Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

O-169

Differentiation, Quantitation, and Stability Assessment of Newly Emergent Kratom Alkaloids in Human Blood via High-Resolution Mass Spectrometry

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Abstract

Introduction: Mitragynine (MG) is the primary psychoactive component of the Kratom plant (*Mitragyna speciosa*). 7-Hydroxy mitragynine (7OH-MG) is also found naturally in the plant but in small amounts (<2%). MG is metabolized to 7OH-MG and further to mitragynine pseudoindoxyl (MP), with the latter conversion possible via enzymatic activity. Lack of federal scheduling and unregulated markets through gas stations, smoke shops, and online retailers have resulted in the proliferation of semi-synthetic formulations of these "Kratom" alkaloids. Products containing 7OH-MG, MP, and dihydro-7-hydroxy mitragynine (DH-7OH-MG, aka MGM-15) pose concern due to their greater potencies compared to MG and have been increasingly implicated in fatalities and intoxications. These alkaloids present complex analytical and interpretive challenges due to their isomeric forms, unsuitability with certain extraction and instrumental techniques promoting analyte interconversion, instability in blood, and limited quantitative information.

Objectives: This presentation describes a quantitative HRMS method for MG, 7OH-MG, MP, and DH-7OH-MG in blood which eliminates analyte interconversion and separates isomers, along with stability data from 12-16 weeks and concentration data from over 200 authentic cases.

Methods: Blood samples were prepared via protein precipitation with acetonitrile and analyzed via liquid chromatography quadrupole time-of-flight mass spectrometry (LC-QTOF-MS) using non-targeted acquisition. Targeted data processing was used for identification and quantitation over a calibration range of 1-1000 ng/mL. Fortified stability samples were prepared in prescreened NaF-KOx preserved human blood and analyzed in triplicate at 14-15 timepoints across 84-112 days, stored by refrigeration (0-10°C) between samplings.

Results: 205 authentic specimens were analyzed. A subset (n=139) was identified to exclude those where MG was the suspected primary or co-ingested alkaloid. Categorized as true 7OH-MG/MP cases, these had a ratio of MG to (7OH-MG + MP) less than one. Mean concentrations were 25±53 ng/mL for MG (median: 9.2 ng/mL, range: 0-430 ng/mL), 146±477 ng/mL for 7OH-MG (median: 58 ng/mL, range: 0-5,000 ng/mL), and 194±239 ng/mL for MP (median: 120 ng/mL, range: 4.8-1,900 ng/mL). In fortified samples, MG and DH-7OH-MG did not decrease by more than 20% over 84 days. Over 112 days, MP also remained stable and did not decrease by more than 10%. 7OH-MG decreased by 89% over 112 days with concurrent increase in MP, demonstrating the conversion of 7OH-MG to MP.

Discussion: MP is highly recommended for inclusion in testing scopes due to its stability and high prevalence in postmortem casework. Significant overlap exists among 7OH-MG/MP cases due to both enzymatic breakdown and cooccurrence in popular product formulations. MP can extend the detection window for 7OH-MG, but time, storage conditions, and scene information must be considered for accurate interpretation. Even without drug product information or testing,

thereby making it difficult to determine if 7OH-MG, MP, or a combination of the two was ingested based on concentrations alone, the presence of one or both alkaloids is still likely causative in nature due to their strong agonism at μ-opioid receptors. Case histories describe presentations characteristic to opioid overdose, including the “foam cone” and pulmonary edema. Continued vigilance is needed as the Kratom alkaloid product landscape evolves and manifests in forensic toxicology casework.

Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

O-170

Phenazolam Emergence: A Case Series of Fatal and Nonfatal Exposures

Brianna Stang[1], Sara Walton[2], Donna Papsun[3], Alex Krotulski[2], Barry Logan[2,3]

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Abstract

Introduction: Novel benzodiazepines are an evolving subclass of novel psychoactive substances (NPS), which are synthetically manufactured with structures resembling other traditional benzodiazepines (e.g. alprazolam, diazepam, clonazepam). Novel benzodiazepines are often sold as counterfeit pills and are increasingly found mixed with opioids. While novel benzodiazepines have CNS depressant effects similar to those of their traditional counterparts, the literature lacks interpretive data on many of these drugs. Consequently, novel benzodiazepines present risks to people who use drugs, and interpretive challenges to forensic pathologists and clinicians, particularly when they are present in drug materials alongside other CNS depressants (i.e. opioids, benzodiazepines) due to synergistic sedative effects, and increased risk of overdose. Based on data from the Center for Forensic Science Research and Education (CFSRE), bromazolam has remained the most prevalent NPS benzodiazepine in recent years despite its placement into Schedule IV under the Convention on Psychotropic Substances Act of 1971. Notably, its positivity has decreased since this legislative action occurred in 2024. For these reasons, another benzodiazepine is expected to replace bromazolam in this market. In recent years, phenazolam detections have increased, making it a strong candidate as the next novel benzodiazepine.

Objectives: This presentation describes the evolution of the NPS benzodiazepine market, highlighting more recent changes due to scheduling actions. Due to its increasing prevalence, five recent case examples involving phenazolam intoxications are presented. Toxicological data is presented along with case histories, though drug chemistry data is available in one case.

Methods: Case histories and autopsy results, where applicable, will be presented with quantitative toxicological data and drug comorbidities identified. Toxicological samples were sent to the CFSRE by forensic agencies and stored in the refrigerator (4°C) until analysis. Samples were analyzed via liquid chromatography quadrupole time-of-flight mass spectrometry (LC-QTOF-MS) using a previously validated broad-spectrum drug screening method. Data were processed against an in-house library containing >1,200 analytes including traditional drugs, therapeutics, adulterants, and NPS. Quantitative results were acquired via multiple validated assays using liquid chromatography-tandem-mass spectrometry (LC-QQQ-MS) and LC-QTOF-MS.

Results: The cases selected for this presentation encompass a variety of analytical findings and case scenarios. These cases involved fatal (n=4) and nonfatal (n=1) exposures to phenazolam throughout North America. Three of the fatalities had cause (phenazolam intoxication: n=2, nonischemic cardiomyopathy: n=1) and manner (suicide: n=1, accident: n=2) of death data available. Phenazolam was detected in toxicological specimens alone and in combination with other drugs such as opioids and benzodiazepines. Newly emerged NPS such as *N*-propionitrile chlorphine and mitragynine alkaloids (e.g., 7-hydroxy mitragynine, mitragynine pseudoindoxyl) were also detected in these cases.

Discussion: Since scheduling action has been taken to control bromazolam, the next novel benzodiazepine to dominate the market remains in question. This case series demonstrates specific scenarios regarding phenazolam use in North America and demonstrates its rise in popularity. While phenazolam was detected alone in some specimens, it was found alongside other benzodiazepines and opioids including other NPS in poly-drug intoxications, highlighting its forensic relevance. Overall, this presentation highlights various examples surrounding phenazolam use as its prevalence continues to increase.

Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

O-171

Toxicokinetics of Four *N*,*N*-Dimethyltryptamine Derivatives Studied in Human *in Vitro* Systems and *in Vivo* by Means of Zebrafish Embryos

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Abstract

Introduction: Seizures of *N*,*N*-dimethyltryptamine (DMT) are frequently reported across Europe and intoxications related to the consumption of tryptamine derivatives are increasing. A novel DMT derivative, DMT-Boc (tert-butyl-3-[2-(dimethylamino)ethyl]indole-1-carboxylate), recently appeared in the market as a potential prodrug of DMT without any information on its toxicokinetics and metabolic fate.

Objectives: The aim of the current study was to investigate the toxicokinetics of DMT-Boc and three prophetic DMT prodrugs, by name DMT-isopropylcarbamate (propan-2-yl 3-[2-(dimethylamino)ethyl]-1H-indole-1-carboxylate), DMT-pivaloylamide (1-{3-[2-(dimethylamino)ethyl]-1H-indol-1-yl}-2,2-dimethylpropan-1-one), and DMT-THP (*N*,*N*-dimethyl-2-[1-(oxan-2-yl)-1H-indol-3-yl]ethan-1-amine).

Methods: Fifty µM of DMT-Boc, DMT-isopropylcarbamate, DMT-pivaloylamide, or DMT-THP were incubated with pooled human liver S9 fraction (pHLS9) for 1 and 6 h. Zebrafish embryos (ZE, 96 h post fertilization) were exposed via bathwater for 24 h at 28 °C with 75 µM DMT-Boc or 100 µM DMT-isopropylcarbamate, DMT-pivaloylamide, or DMT-THP and extracted with methanol after lyophilization. Additionally, ten CYP isoenzymes and flavin-containing monooxygenase 3 (FMO3) were incubated for 30 min to assess their involvement in drug metabolism and monoamine oxidase (MAO) involvement was investigated through MAO-A and MAO-B 6h incubations. In vitro metabolic stability was evaluated through human liver microsomes (pHLM) incubations stopped at different time points (0-180 min) and monitoring of parent compound depletion. Plasma protein binding (PPB) was evaluated in fresh plasma by calculating the unbound fraction (f_u). All analyses were performed via reversed-phase liquid chromatography coupled to high-resolution tandem mass spectrometry in full scan and data-dependent MS^2 mode with an inclusion list of mass-to-charge ratios of expected metabolites.

Results: Following in vitro and in vivo metabolism studies, *N*-demethylation, *N*,*N*-bis-demethylation, hydroxylation, and glucuronidation resulted to be the predominant biotransformations. The monooxygenases activity screening suggested that *N*-demethylated metabolites were produced by numerous CYP isoforms. At least three CYP isoforms and FMO3 participated in the formation of the *N*-oxides. After DMT-THP incubations with MAO-A additional metabolites were detected (oxidative deamination and oxidative deamination with reduction). DMT-Boc, DMT-isopropylcarbamate, and DMT-pivaloylamide displayed in vitro

metabolic half-lifes between 74 and 79 min, DMT-THP >180 min. PPB calculated for DMT-Boc, DMT-isopropylcarbamate, and DMT-THP was around 99%, and 100% for DMT-pivaloylamide.

Discussion: The combined in vivo and in vitro approaches allowed the comprehensive investigation of the toxicokinetics of four DMT derivatives. Since many monooxygenases contributed to the initial phase I metabolic steps, significant drug–drug interactions or interindividual variability influencing their metabolism are considered unlikely. Following MAO-A incubation additional metabolites were detected for DMT-THP confirming oxidative deamination. The half-lives of DMT-Boc, DMT-isopropylcarbamate, and DMT-pivaloylamide were lower than reported for DMT, while DMT-THP exceeded 180 minutes. This is consistent with MAO assay results indicating a significant role of MAO, particularly MAO-A, in DMT-THP metabolism. High PPB and increased lipophilicity suggest limited free fraction but potential displacement interactions. Compound-specific metabolites were detected for each substance, which may be used in the future to differentiate between the intake of the prodrug and DMT, also in combination with the presence of the parent compound.

Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

O-172

Dihydro-7-Hydroxy Mitragynine (MGM-15) – A Novel Synthetic Kratom Alkaloid Implicated in Fatalities

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Abstract

Introduction: The United States has observed a rise in smoke shops and online marketplaces selling kratom and related drug products. While semi-synthetic kratom alkaloids, like 7-hydroxy mitragynine and mitragynine pseudoindoxyl, have increased in availability and product diversity, so too have synthetic kratom alkaloids, most notably dihydro-7-hydroxy mitragynine (DH-7OHMG, also referred to as "MGM-15"). Synthetic kratom alkaloids are more potent than mitragynine and, similar to other synthetic novel psychoactive substances (NPS), offer numerous opportunities for structural variations. DH-7OHMG is reported to be 30x more potent than mitragynine. Today, DH-7OHMG is available from online retailers, now marketed under the acronym "DHM".

Objectives: This presentation will highlight the emergence of DH-7OHMG in the recreational drug supply, showcasing test results from toxicology specimens and drug product analysis. A review of medicolegal death investigations will be included to demonstrate implications in fatal drug overdoses.

Methods: Biological specimens (e.g., blood) from medicolegal death investigations were submitted by partnering medical examiner or coroner offices and forensic toxicology laboratories. Comprehensive toxicological analysis was performed by liquid chromatography quadrupole time-of-flight mass spectrometry (LC-QTOF-MS) using non-target acquisition and targeted data processing for the presence of more than 1,200 targets. Quantitative analysis for DH-7OHMG was also performed by LC-QTOF-MS. Samples were prepared using protein precipitation. The calibration range of the assay was 1-1,000 ng/mL. The internal standard was fentanyl-D5. The instrument used was a Sciex X500R QTOF-MS coupled with a Sciex Exion UPLC. Qualitative and quantitation data were processed using SciexOS. The runtime of the assay was 15.5 mins. The method was validated in accordance with ASB 036 Standard Practices for Method Validation in Forensic Toxicology.

Results: DH-7OHMG was first identified in drug products submitted to our laboratory in August 2025 labeled as "MGM-15". The first toxicology case testing positive for DH-7OHMG was associated with a death that occurred in September 2025, demonstrating the rapid emergence of this new drug. In 17 cases, the average DH-7OHMG blood concentration was 320±660 ng/mL (median: 170 ng/mL, range: 50-2,900 ng/mL); in these same cases, the average mitragynine blood concentration was 95±100 ng/mL (median: 62 ng/mL, range: 3.2-340 ng/mL). Our first case containing DH-7OHMG was from New Jersey and involved an individual purchasing research chemicals. The blood concentration of DH-7OHMG was 2,900 ng/mL, in addition to mitragynine (340 ng/mL), lorazepam (19 ng/mL), delorazepam (100 ng/mL), and meprobamate (3,100 ng/mL). A recent case from Florida was submitted in March 2026 and involved an individual with history of prior overdose and "kratom" consumption. The blood concentration of DH-7OHMG was 190 ng/mL, in addition to mitragynine (260 ng/mL), clonazepam (3.9 ng/mL), 7-aminoclonazepam (51 ng/mL),

venlafaxine (210 ng/mL), and O-desmethylvenlafaxine (290 ng/mL). The manner of death was accident, and the cause of death was combined acute DH-7OHMG and mitragynine toxicity.

Discussion: DH-7OHMG is the newest kratom alkaloid in an evolving landscape of these synthetic substances. DH-7OHMG quickly emerged among recreational drug markets and has been involved in deaths across several states. With increased potency compared to mitragynine, forensic toxicology must remain aware of DH-7OHMG and other synthetic kratom alkaloids.

Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

Conflict of Interest

Grant - NIJ

O-173

The Pharmacokinetics of Clonazafone and Its Analytical Challenges – an In-Vivo Experiment

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Abstract

Introduction: Clonazafone (glycyl-*N*-[2-(2-chlorobenzoyl)-4-nitrophenyl]-glycinamide) is a new psychoactive substance that is rapidly metabolized by proteases to desglycylclonazafone. The latter compound exists in a pH-dependent equilibrium with clonazepam. Since clonazepam, unlike the designer benzodiazepine clonazafone, is an approved medical drug, different legal regulations apply. Therefore, distinguishing between the two substances poses a significant forensic challenge.

Objectives: To investigate the pharmacokinetics, a clonazafone self-administration study was conducted (n=3). The study also aimed to test a previously proposed hypothesis that the intake of clonazafone can be distinguished from that of clonazepam based on the presence of the marker metabolite 2-amino-2'-chloro-5-nitrobenzophenone in the blood. To date, this metabolite has not been detected in clonazepam samples.

Methods: Three volunteers ingested 20 mg of clonazafone in a gelatin capsule. Whole blood stabilized with sodium fluoride was collected before ingestion and again after 0.25, 0.5, 0.75, 1, 1.5, 2, 3, 5, 7, 10, and 20 hours (total whole blood volume: ca. 108 mL per person). To capture the desglycylclonazafone-clonazepam equilibrium, the blood was aliquoted immediately after collection and directly frozen at -20 °C. Sample preparation included protein precipitation at native pH with acetonitrile (1:2, v/v), evaporation, and reconstitution with 10 mM ammonium formate:acetonitrile (95:5) containing 0.1% formic acid (corresponding to the liquid chromatography (LC)-starting conditions). Reconstitution was performed within 3 hours before injection to limit equilibrium shifts induced by acidic pH. Sample extracts were analyzed by LC-high resolution mass spectrometry (HRMS; reversed-phase chromatography, gradient elution; Sciex ZenoTOF 7600, untargeted data acquisition, DDA mode, MS1 scan range m/z 100-1000, MS2 m/z 50-1000).

To evaluate accuracy and precision, calibration samples and quality control samples for clonazepam, desglycylclonazafone and clonazafone (concentration-range 0.1-100 ng/mL) were measured using clonazepam-*d*4 and trimipramine-*d*3 as internal standards.

Results: Clonazafone reached its maximum blood concentration in the 3 volunteers 0.75, 0.5, and 0.75 hours after administration and remained detectable for 1.5, 1.5, and 2 hours. Desglycylclonazafone reached its peak concentration after 0.75 hours in all cases and remained detectable for 3, 5, and 10 hours, whereas clonazepam reached its maximum concentration after 1, 1.5, and 1.5 hours and remained detectable throughout the entire 20-hour observation period. Significant instability of clonazafone and desglycylclonazafone was observed in the spiked calibration and quality control samples. This instability was strongly influenced by the time from thawing to protein precipitation (speed of sample preparation), suggesting a potential impact on the detection windows and posing a challenge to the

quantitative results. The potential marker metabolite 2-amino-2'-chloro-5-nitrobenzophenone was detected from 0.25-0.75 hours post ingestion and remained positive in all participants until the final sampling time point.

Discussion: The pronounced instability (rapid metabolism and chemical equilibrium) of clonazafone and desglycylclonazafone significantly limits their forensic detectability and can make it difficult to directly confirm clonazafone use. However, the sustained presence of 2-amino-2'-chloro-5-nitrobenzophenone enables differentiation between clonazafone and clonazepam use, highlighting its value as a robust forensic marker despite the limited sample size of this exploratory study. Major analytical challenges related to storage, sample preparation, and quantification underscore the need for careful consideration and further evaluation.

Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

O-174

Development of a Targeted Dynamic Analytical Strategy for the Detection of New Psychoactive Substances in Forensic Toxicology

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Abstract

Introduction: The increasing development of new psychoactive substances (NPS) has created an ongoing analytical challenge for clinical and forensic toxicology laboratories. Furthermore, their identification in biological samples is of great interest to toxicologists, as it makes it possible to assess the spread of NPS within the population, diagnose intoxications, and evaluate harm resulting from the use of these substances. There is a demand for analytical screening methods that are continuously updated and include multiple drugs, among them NPS. In this context, a dynamic targeted approach was developed, in which the analytical panel is continuously updated through the incorporation of newly emerging NPS. New analytes are continuously acquired through reference standards or in-house standards, obtained by purification and characterization of compounds isolated from seized materials. Following optimization of LC-MS/MS parameters and a validation method, the new analytes are incorporated into the screening panel. This strategy enables continuous adaptation of the method to changes in the illicit drug market while promoting integration between drug seizure data and forensic toxicology analyses of biological samples.

Objectives: This project aims to develop a qualitative targeted screening method for determination of NPS using liquid chromatography–mass spectrometry (LC-MS/MS) associated with a dynamic analytical strategy.

Methods: For sample preparation, 250 µL of postmortem blood and 25 µL of internal standard (deuterated cocaine, 100 ng/mL) were mixed with 1 mL of iced acetonitrile. Samples were mixed by tube inversion and orbital shaking, centrifuged at 14000 rpm for 10 min, and 500 µL of the supernatant was transferred to an autosampler vial. Analyses were performed using a Shimadzu LC-MS/MS 8045 equipped with an Atlantis® T3 column (100 Å, 3 µm, 3 × 150 mm). The mobile phase consisted of water containing 0.1% formic acid and 2 mmol/L ammonium formate (A) and acetonitrile (B). The flow rate was 0.400 mL/min and the run time was 16.5 min. Thus far, the qualitative method comprises forty-four NPS with limits of detection (LODs) ranging from 5 to 200 ng/mL in postmortem blood.

Results: Among 259 postmortem blood samples received from the Scientific and Technical Police of the State of Sao Paulo, twenty-four tested positive for MDMB-4en-PINACA. In one MDMB-4en-PINACA-positive sample, protonitazene was simultaneously identified. Additionally, four samples tested positive for MDMB-CHMINACA.

Discussion: The high number of positive cases for MDMB-4en-PINACA is consistent with findings from the 2024 São Paulo Report on New Psychoactive Substances. Of the 3,381 NPS seizures recorded, MDMB-4en-PINACA was identified in 1,579 cases, making it the most prevalent NPS among seized substances. Notably, protonitazene and MDMB-CHMINACA were included in

the analytical panel using in-house standards produced from seized materials. Their successful detection in postmortem blood illustrates the value of converting information obtained from drug seizures into analytical tools for forensic toxicology. In conclusion, this dynamic targeted approach proved capable of determining NPS in postmortem blood and provides a structured framework for the continuous incorporation of newly emerging substances, ensuring adaptation to changes in the illicit drug market.

Acknowledgments

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Disclosure

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O-175

Detection of Etomidate and Its Metabolites in Human Hair

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Abstract

Introduction: Etomidate, originally an intravenous anesthetic, has emerged as a novel new psychoactive substance (NPS) in Singapore's forensic casework since its first detection in "Kpods" e-vaporizers in Q3 2024. Subsequent urinary detections increased markedly from October 2024, establishing etomidate as the dominant NPS despite its short urinary detection window. Hair testing offers an extended detection window and is useful for monitoring drug consumption patterns.

Objectives: This study describes an analytical approach for the identification of etomidate, its metabolite etomidate acid, and related analogues in drug users' hair using high resolution mass spectrometry for screening and liquid chromatography-tandem mass spectrometry (LC-MS/MS) for confirmatory analysis.

Methods: For screening, 20 mg of hair was pulverized in acidified methanol, extracted using supported liquid extraction (SLE), and analyzed using a Thermo Scientific Vanquish LC system coupled to an Orbitrap Exploris 120 mass spectrometer (LC-Orbitrap MS) equipped with a heated electrospray ionization (HESI) source operating in positive ionization mode. Data were acquired in full scan data-dependent MS^2 (FS-ddMS^2) mode with inclusion list. For confirmatory analysis, 20 mg of hair was pulverized in sodium phosphate buffer (pH 6), extracted using solid-phase extraction (SPE) and analyzed using an Agilent 6495C iFunnel triple quadrupole LC-MS/MS system in multiple reaction monitoring (MRM) mode with positive electrospray ionization. Confirmation was based on a qualitative method employing predefined cut-off concentrations.

Results: The methods were validated according to ANSI/ASB Standard 036 for Method Validation in Forensic Toxicology. Screening limits of detection (LODs) were 50 pg/mg for etomidate and 100 pg/mg for etomidate acid, while confirmatory LODs were 70 pg/mg and 50 pg/mg, respectively. A confirmatory cut-off concentration of 100 pg/mg was established for etomidate. Matrix effects ranged from -7.1% to 7.3 % for etomidate and from -43.8% to 58.7% for etomidate acid, with greater variability observed for the metabolite due to the absence of a deuterated internal standard (IS). Precision and accuracy met the acceptance criteria, except for a higher intra-day CV (28.2%) for etomidate acid, which was attributed to the use of a non-deuterated analogue as the IS. No significant interferences were observed from common drugs of abuse, structurally related compounds, or endogenous matrix components. Extracted samples remained stable for at least seven days at 4°C and 15°C.

Discussion: Correlation studies using 25 authentic hair samples from etomidate users demonstrated the method's capability to detect both etomidate and its metabolite, with no false-positives or false-negatives observed at the confirmatory cut-off concentration of 100 pg/mg. However, result interpretation remains challenging. Etomidate acid was consistently detected at substantially lower concentrations than etomidate. In addition, the mean wash-to-hair ratio for etomidate was approximately 30%, with some samples exhibiting ratios as high as 90%, suggesting potential external contamination. These observations limit the use of the metabolite

as supportive evidence of consumption and complicate reliance on metabolite-to-parent ratios as interpretative tools. Overall, the findings highlight the analytical and interpretative complexities of etomidate hair analysis and underscore the need for careful case-by-case evaluation.

Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

O-176

A Comprehensive LC-MS/MS Triage Workflow for 135 Forensic Targets in Qatar: Optimizing Solid-Phase Extraction for the Preservation of Highly Labile Ester-Linked Synthetic Cannabinoids

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Abstract

Introduction: The rapidly evolving New Psychoactive Substances (NPS) landscape necessitates adaptable, comprehensive analytical approaches for routine forensic casework in Qatar. This study aims to develop, validate, and implement an immunoassay-guided triage protocol and a consolidated Solid-Phase Extraction (SPE) workflow coupled with liquid chromatography-tandem mass spectrometry (LC-MS/MS). This method dynamically integrates 135 targets (parents and selected metabolites)—encompassing emerging NPS, traditional drugs of abuse, and Therapeutic Drug Monitoring (TDM) targets—into a unified human urine screening panel.

Objectives: A key objective, representing the method's core novelty, is to establish specialized extraction parameters to prevent artifactual degradation, thereby enabling routine detection and semi-quantification of highly labile, intact synthetic cannabinoid (SC) parent compounds.

Methods: The method employs an immunoassay-guided triage decision matrix to efficiently route urine samples, prioritizing a unified Solid-Phase Extraction (SPE) workflow thoroughly validated per ANSI/ASB guidelines. This SPE framework—utilizing EVOLUTE® ABN cartridges, surrogate internal standards, and a shared calibration curve—encompasses Streams A and B. As the primary focus, Stream A is dedicated to SC-positive samples (e.g., AB-PINACA ≥3 ppb cut-off); it strictly excludes enzymatic treatment and employs optimized buffer, pH, and temperature controls to prevent artifactual degradation. Stream B applies enzymatic hydrolysis for conjugated targets. Lastly, Stream C employs a rapid "dilute-and-shoot" approach, with its own specific calibration curve, for non-conjugated targets and immunoassay-negative samples; crucially, it serves to demonstrate that intact SC parents cannot be recovered without Stream A's optimized SPE. For poly-drug cases, samples requiring both SPE streams are split into aliquots, ensuring Stream B's harsh hydrolysis does not destroy the fragile ester-linked NPS preserved in Stream A.

Results: The LC-MS/MS methodology yielded analyte-dependent Limits of Detection (LODs) reflecting the panel's diverse chemistry. Most of the 135 analytes achieved LODs of 1–10 ppb; although highly polar gabapentinoids (e.g., pregabalin, gabapentin, mirogabalin) exhibited higher LODs (up to 50 ppb), all values remained forensically relevant. The comparative evaluation showed that the polymer-based EVOLUTE® ABN sorbent effectively addressed the extraction challenges of large, multi-class panels, ensuring consistent recovery across diverse targets. Crucially, Stream A's optimized conditions significantly better preserved highly labile SCs (e.g., MDMB-4en-PINACA, 5F-ADB, ADB-BUTINACA, ADB-4en-PINACA, MDMB-INACA, MDMB-BUTINACA). Conversely, Stream C's dilute-and-shoot approach failed to recover these intact parents, while Stream B's enzymatic hydrolysis artifactually degraded these ester-linked targets, favoring metabolite formation. By rescuing these fragile structures, unified calibration

allowed accurate semi-quantification, enabling unprecedented routine detection of intact SC-parents at high concentrations in authentic human urine, challenging traditional reliance on SC-metabolite screening.

Discussion: This validated triage workflow demonstrates Qatar's toxicology laboratory's capability to address complex forensic challenges through a structured approach. Primarily, it establishes a robust, operationally viable protocol capable of simultaneously managing 135 diverse analytes via a unified calibration strategy, optimizing laboratory throughput and reagent efficiency. Concurrently, it addresses the unique vulnerabilities of the modern NPS market; by strategically bypassing harsh hydrolysis in favor of optimized extraction parameters on polymer-based EVOLUTE® ABN sorbents, the method rescues highly labile SCs from artifactual degradation. Ultimately, this consolidated methodology offers a practical blueprint for global forensic laboratories to maximize detection accuracy and definitively mitigate false-negative reporting risks in modern casework.

Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

O-177

BTMPS in Biological Samples in the Absence of Illicit Drugs

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Abstract

Introduction: BTMPS testing in forensic toxicology casework began after it was discovered that it was being cut into illicit drugs. BTMPS, (Bis[2,2,6,6-tetramethyl-4-piperidyl] sebacate), brand name Tinuvin®770, is commonly used in the manufacture of both food and medical grade plastics as a UV-stabilizing additive. While BTMPS is often found in samples alongside fentanyl, synthetic opioids, and stimulants, it can also be found in the absence of illicit drugs.

Objectives: To appreciate that BTMPS can be found in casework without the presence of illicit drugs and how this may effect case interpretation. Also possible sources of BTMPS in licit drug cases will be discussed.

Methods: Blood specimens were extracted via protein precipitation and screened by LC-QToF-MS for over 350 drugs and metabolites. Confirmation was done on a second aliquot of the sample on the LC-QToF-MS.

Results: Two positive cases with BTMPS without the presence of illicit drugs, and four cases with the presence of illicit drugs have been identified to date. The cases without illicit drugs were an oxycodone overdose, and an amphetamine overdose. Both decedents had empty prescription bottles found at the scene. A third case was identified involving an individual that was subject to urine testing due to pain management therapy. The urine test results matched the prescriptions the individual was taking with no illicit drugs present, therefore he was considered compliant. However BTMPS was also detected in the urine sample.

Discussion: How does BTMPS enter the licit drug supply? It is possible that the BTMPS was a contaminant on the pills from the plastic medication bottle(s) in which they were stored since manufacture, and because so many of them were ingested at one time, the BTMPS accumulated to a detectable concentration. For the individual in pain management therapy, he was on multiple medications most of which were stored in plastic bottles, including an opioid.

University of Washington drug detection data showed that BTMPS was detected in licit drug samples. In 311 prescription opioid samples (excluding fentanyl), 3.5% were positive for BTMPS, thus supporting the BTMPS leaching out of the plastic pill containers. Further evidence that the BTMPS may be leaching out of plastic was found. BTMPS is a potent calcium channel blocker and nicotinic receptor antagonist. For this reason it has been used in nicotinic receptor studies. It was discovered by Papke et.al, 1994, that the control and the BTMPS treated nicotinic receptors produced the same results of receptor blockade. It was determined that BTMPS was leaching out of the plastic syringes that were being used. When the plastic syringes were removed from use, the control and BTMPS groups no longer produced the same results.

Because BTMPS has been found in biological specimens from postmortem and ante-mortem samples without the presence of illicit drugs, in prescription opioid pills, and found to leach out of plastic syringes, there can be no assumption made that an individual used illicit drugs if BTMPS is detected.

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Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

O-178

Integrating Predictive Fragmentation Into HRMS Workflows for the Identification of Metabolite Biomarkers Phase I and Phase II and the Extension of Detection Windows in Forensic Toxicology

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Abstract

Introduction: The detection of new psychoactive substances (NPS) in forensic toxicology is often limited by rapid metabolism, instability and low concentrations of parent compounds in biological samples.

Phase II metabolites, particularly glucuronides, are valuable biomarkers due to their abundance and persistence, enabling extended detection windows. However, their identification remains challenging because of the limited availability of reference standards and the complexity of non-targeted in High Resolution Mass Spectrometry (HRMS) data.

For unstable or rapidly metabolized substances, Phase I metabolites also represent important biomarkers, especially when the parent compound is no longer detectable. Furthermore, their characterization can support the prediction and annotation of corresponding Phase II metabolites. This is particularly relevant for synthetic cathinones, where more stable Phase I metabolites, such as dihydro-metabolites, can serve as complementary biomarkers.

Therefore, the combined characterization of Phase I and Phase II metabolites, together with the integration of predictive fragmentation strategies into validated HRMS-based workflows, represents a promising approach to improve substance identification.

Objectives:

This study aimed to:

1. Develop and validate more than of 200 drugs in biological samples
2. Integrate predictive fragmentation strategies to improve metabolite identification *phase I and II*.
3. Incorporate these strategies into HRMS workflows processed to enhance non-targeted and retrospective screening.

Methods: A qualitative UHPLC-HRMS/MS acquisition method was developed using a dilute-and-shoot approach for urine samples. The workflow combined both targeted and non-targeted data analysis strategies [1].

Method validation included the evaluation of selectivity, sensitivity, limit of detection (LOD), limit of identification (LOI), matrix effects and carryover.

A predictive data processing strategy was implemented based on a predictive mass list, combined with the characteristic neutral loss of glucuronic acid (–176.0321 Da), diagnostic fragment ions, accurate mass filtering and isotopic pattern recognition.

Additionally, complementary fragmentation pathways were incorporated to support the identification of structurally related metabolites through diagnostic ions and neutral losses [2]. In this context, dihydro-metabolites *for cathinones* were characterised using LC-HRMS (Orbitrap and qTOF) as described by Menéndez-Quintanal et al. [3].

Results: The validated HRMS workflow demonstrated high selectivity and sensitivity for the analytes studied, as well as for the identification of glucuronide metabolites in urine samples.

The application of predictive filtering strategies enabled a significant reduction in data complexity, allowing prioritisation of relevant glucuronide candidates. Identification was achieved through detection of characteristic neutral losses, confirmation via diagnostic fragment ions and consistency with predicted fragmentation patterns.

The incorporation of predictive fragmentation knowledge improved the detection capability of unknown or previously unreported metabolites and enhanced confidence in structural elucidation, particularly in the absence of reference standards.

Glucuronides showed improved detectability compared to parent compounds, supporting their role in extending detection windows.

Discussion: This study demonstrates that validated HRMS workflows integrating predictive fragmentation significantly improve the identification of glucuronide metabolites in forensic toxicology.

The combination of methodological validation with predictive data analysis ensures robust, reproducible and reliable metabolite identification in complex biological matrices.

Furthermore, the inclusion of stable Phase I metabolites as complementary biomarkers enhances detection capability, particularly for unstable NPS.

This integrated approach enables both prospective and retrospective screening and represents a powerful strategy for addressing current challenges in forensic toxicology

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Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

O-179

When Structure Matters: Immunoassay Cross-Reactivity of Synthetic Cocaine Derivatives

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Abstract

Introduction: Immunoassays are widely used as rapid qualitative and semi-quantitative screening tools for detecting drugs of abuse or their metabolites in biological matrices such as urine, saliva, and blood. With the emergence of synthetic cocaine derivatives as new psychoactive substances (NPS), immunoassays face new analytical challenges but also offer opportunities for broader detection. Structurally modified cocaine analogues of the phenyltropane class may exhibit altered antibody binding due to subtle chemical and stereochemical variations, including (–)-2β,3β-, 2α,3β-, and 2β,3α-stereoisomers, potentially affecting assay sensitivity. In addition, several metabolites of these synthetic cocaine derivatives share structural similarities with benzoylecgonine (BE), the primary urinary marker of cocaine consumption, and can therefore exhibit measurable cross-reactivity in BE-targeting immunoassays. Consequently, these assays may respond not only to BE but also, to varying extents, to cocaine, related metabolites, and certain synthetic analogues, which may facilitate the preliminary detection of cocaine-related NPS during routine toxicological screening.

Objectives: The aim of this study was to systematically evaluate the cross-reactivity of 24 synthetic cocaine derivatives (e.g. troparil, dichloropane, dimethocaine, tropacocaine), including their metabolites and stereoisomers, in commonly used urine-based BE-targeting immunoassays.

Methods: The selected compounds were assessed for cross-reactivity using four widely applied urine immunoassays: CEDIA® cocaine test (Thermo Fisher Scientific), Syva® EMIT® Cocaine Assay (Siemens), Roche® DAT immunoassay for cocaine metabolite, and PIA® single-parameter lateral flow test (Protzek). Compounds were initially tested at concentrations of 10, 100, and 1000 ng/mL. Compounds exceeding the respective assay-specific BE cutoff values (CEDIA: 25 ng/mL-IA; EMIT: 150 ng/mL-IA; Roche DAT: 25 ng/mL-IA; PIA: 200 ng/mL-IA) were subsequently analyzed in greater detail to determine and quantify cross-reactivity.

Results: Despite their structural similarity to cocaine, the majority of the tested compounds exhibited no detectable activity in the investigated immunoassays, underscoring the high analytical specificity of BE-targeting assays. Accordingly, none of the compounds exceeded the cutoff value of the Roche DAT immunoassay. In the Syva® EMIT® cocaine assay, the (–)-2β,3β-stereoisomers of the metabolites troparil acid and dichloropane acid exceeded the cutoff value at the highest tested concentration, resulting in cross-reactivities of 23% and 16%, respectively. In contrast, the corresponding 2β,3α-stereoisomers did not show any cross-reactivity. In the CEDIA immunoassay, the 2β,3β-stereoisomer of tropacocaine exceeded the cutoff value at the highest tested concentration, corresponding to a cross-reactivity of approximately 9%. Consistently, the PIA® lateral flow test yielded also showed a positive result for the 2β,3β-stereoisomer of tropacocaine at the highest tested concentration.

Conclusion: BE-targeting immunoassays exhibited high specificity, with most synthetic cocaine derivatives and their metabolites showing no measurable cross-reactivity. Limited, stereochemistry-dependent cross-reactivity was observed for selected 2β,3β-isomers. These findings indicate a risk of negative results in routine immunoassay screenings and highlight the need for complementary analytical methods for the detection of synthetic cocaine derivatives.

Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

O-180

Potent and Irreversible? Evaluating the Monoamine Oxidase Inhibition Profile of Eight Psychedelics of the Cyclopropylamine Class

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Abstract

Introduction: Novel compounds are continuously introduced to the global drugs of abuse market without preclinical studies making pharmacological effects and risk of drug interactions hard to predict. 2,5-Dimethoxy-4-methylphenylcyclopropylamine (DMCPA) is a known serotonin receptor agonist and potent psychedelic. As a cyclopropylamine (CPA), it is also closely related to the antidepressant and irreversible dual monoamine oxidase (MAO) inhibitor tranylcypromine. MAO inhibition (MAOI) might contribute to the psychedelic activity but is also associated with monoaminergic toxicity due to elevated serotonin, (nor)adrenaline, and dopamine levels.

Objectives: This study aimed to assess the risk of intoxication due to MAOI after consumption of DMCPA or seven DMCPA derivatives by comparing their in vitro potency with tranylcypromine, other relevant single MAO inhibitors (alpha-methyltryptamine, 5-(2-aminopropyl)indole, selegiline), and related drugs of abuse of the 2C-series.

Methods: CPAs were categorized by their substitution patterns as follows: halogen, methoxy, or methyl substitution. A previously validated inhibition assay using recombinant human MAO isoforms A or B and kynuramine as substrate followed by hydrophilic interaction liquid chromatography-high-resolution tandem mass spectrometry analysis (Wagmann et al., 2017) was applied. Following statistically significant MAOI during prescreening (10µM inhibitor concentration), IC50 values were determined by plotting the relative metabolite concentration formed over the logarithm of the inhibitor concentration (10 different inhibitor concentrations, each). Tranylcypromine was used as positive control and for comparison. Additionally, the effect of a 30min preincubation (inhibitor + MAO) before kynuramine addition was assessed to investigate potential irreversible binding.

Results: All CPAs and tranylcypromine significantly inhibited both MAO isoforms. MAO-A activity decrease ranged from 50% (DMCPA) to 98% (2,4,6-trimethoxyphenylcyclopropylamine), and from 37% (DMCPA and 2,5-dimethoxy-4-bromophenylcyclopropylamine) to 97% (2,4,6-trimethoxyphenylcyclopropylamine) for MAO-B. Tranylcypromine decreased MAO activities by 87% (MAO-A) and 98% (MAO-B). Preincubation fortified MAOI, indicating irreversible inhibition. In the halogen group, lowest IC_{50} values were determined for 2,6-dimethoxy-4-fluorophenylcyclopropylamine (MAO-A: 0.46µM; MAO-B: 1.2µM) and IC_{50} values increased stepwise from 2,5-dimethoxy-4-iodophenylcyclopropylamine (MAO-A: 2.5; MAO-B: 7.7µM) to 2,5-dimethoxy-4-bromophenylcyclopropylamine (MAO-A: 4.2µM; MAO-B: 17µM) to 2,5-dimethoxy-4-chlorophenylcyclopropylamine (MAO-A: 10µM; MAO-B: 24µM). In the methoxy group, IC_{50} values of 2,4,6-trimethoxyphenylcyclopropylamine (MAO-A: 0.16µM; MAO-B: 0.26µM) were lower than those of 2,4,5-trimethoxyphenylcyclopropylamine (MAO-A: 2.1µM; MAO-B: 6.8µM), while in the methyl group, 2,6-dimethoxy-4-methylphenylcyclopropylamine's IC_{50} values (MAO-A: 9.8µM; MAO-B: 9.5µM) were lower than those of DMCPA (MAO-A: 13µM; MAO-B: 33µM). IC_{50} values of tranylcypromine were 0.78µM (MAO-A) and 0.15µM (MAO-B).

Discussion: Low IC_{50} values of 2,4,6-trimethoxyphenylcyclopropylamine and 2,6-dimethoxy-4-fluorophenylcyclopropylamine indicate clinically relevant dual MAOI comparable to tranylcypromine, as well as alpha-methyltryptamine and 5-(2-aminopropyl)indole (0.38μM and 0.20μM, respectively), two drugs of abuse known to be potent MAO-A inhibitors. Nevertheless, selegiline's IC_{50} value (0.017μM), a clinically used selective MAO-B inhibitor, was lower. Compared to related 2C-series drugs of abuse, e.g., 2,5-dimethoxy-4-iodophenethylamine (MAO-A: 125μM; MAO-B: 55μM) or 2,5-dimethoxy-4-methylphenethylamine (only MAO-B: 24μM), the lower IC_{50} values of the CPAs indicated a stronger MAOI and a higher risk of monoaminergic toxicity after consumption. The significant and most likely irreversible inhibition of both MAO isoforms by all CPAs suggest a high risk of a serotonin syndrome or hypertensive crises, especially in combination with serotonin reuptake inhibitors or upon repeated use. Nevertheless, clinical studies or case series would be needed for final evaluation.

Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

O-181

Is “Levamisole” Always Levamisole in Adulterated Cocaine? Answer Through Enantioselective High-Performance Liquid Chromatography and Supercritical Fluid Chromatography Coupled With Tandem Mass Spectrometry

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Abstract

Introduction: Levamisole, an anthelmintic associated with adverse effects such as agranulocytosis and vasculopathy, is commonly reported as a cocaine adulterant. However, evidence suggests that racemic tetramisole, composed of levamisole (S-tetramisole) and dexamisole (R-tetramisole), may be the actual adulterant. Conventional mass spectrometry cannot differentiate these compounds without enantioselective separation, potentially leading to misidentification. In addition, uncertainties remain regarding tetramisole metabolism and the formation of metabolites such as aminorex and 4-phenyl-2-imidazolidinone. Therefore, enantioselective analytical methods are needed for accurate identification of tetramisole, its enantiomers, and related metabolites in biological samples.

Objectives: This study aimed to develop and validate enantioselective high-performance liquid chromatography-tandem mass spectrometry (HPLC-MS/MS) and supercritical fluid chromatography-tandem mass spectrometry (SFC-MS/MS) methods for the separation and quantification of tetramisole enantiomers and their metabolites. The study further sought to determine whether levamisole or racemic tetramisole is the predominant adulterant in cocaine and to investigate the metabolic formation of aminorex and 4-phenyl-2-imidazolidinone in human samples.

Methods: Enantioselective methods were developed using chiral polysaccharide-based stationary phases and optimized for selectivity, resolution, and analysis time on both HPLC-MS/MS and SFC-MS/MS platforms. Method validation included assessment of linearity, precision, accuracy, and sensitivity. The average limits of detection (LOD) for all the biological matrices were 2 ng/mL for racemic tetramisole. A total of 51 biological specimens were analyzed, comprising 25 oral fluid samples, 26 blood samples, and additional post-mortem specimens collected from cocaine users and forensic autopsy cases. Oral fluid and blood samples from cocaine users included paired specimens where available. Samples were analysed for tetramisole enantiomers and the metabolites aminorex, 4-OH-tetramisole, raxamino and 4-phenyl-2-imidazolidinone, and enantiomeric ratios were evaluated to assess the nature of the adulterant and its metabolism.

Results: The analytical methods successfully achieved baseline separation of the enantiomers of tetramisole. All samples contained both levamisole and dexamisole, with average enantiomeric ratio of 1.2 (dexamisole to levamisole). Approximately 60% of the samples displayed a near-racemic composition. Contrary to the common assumption that

levamisole is the predominant cocaine adulterant, these findings suggest that racemic tetramisole may be more prevalent in street cocaine samples. Metabolite analysis revealed that both aminorex and 4-phenyl-2-imidazolidinone, historically attributed to levamisole metabolism, are also produced following tetramisole exposure in humans. Detection of these metabolites in biological specimens further supports their relevance in forensic toxicology. The application of SFC-MS/MS demonstrated advantages including faster analysis times and improved chromatographic resolution, supporting its potential as a cost-effective and environmentally sustainable alternative to HPLC-MS/MS.

Discussion: The findings suggest that racemic tetramisole may be a common cocaine adulterant and that reports of "levamisole" based solely on non-enantioselective MS methods should be interpreted cautiously. Differentiating levamisole from dexamisole improves the accuracy of adulterant identification and toxicological interpretation. The detection of aminorex, 4-OH-tetramisole, raxamino and 4-phenyl-2-imidazolidinone enhances understanding of tetramisole metabolism, while the successful application of SFC-MS/MS demonstrates its potential as an efficient and environmentally sustainable tool for forensic toxicology.

Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

O-182

A Hallucinogenic Tryptamine Panel for the Modern Era and its Application to Forensic Toxicology Casework

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Abstract

Introduction: Tryptamines are a diverse drug class known for their hallucinogenic effects. The most commonly encountered tryptamine in forensic toxicology is 4-hydroxy-*N*,*N*-dimethyltryptamine (4-OH-DMT), or psilocin. Psilocin is the active metabolite of psilocybin, which occurs naturally in "magic mushrooms". Recently, mushroom-related edibles claiming to contain natural mushroom extracts have become increasingly available at vape shops and in the online marketplace. External agencies also reported the presence of 4-acetoxy-*N*,*N*-dimethyltryptamine (4-AcO-DMT), a prodrug of psilocin, in some of these products. During this time, the Armed Forces Medical Examiner System, Division of Forensic Toxicology Laboratory (AFMES-DFT) saw a rising number of cases involving mushroom gummies and other edibles. This led to the development of an updated analytical method to detect the presence of synthetic tryptamines in forensic toxicology casework.

Objectives: This presentation discusses challenges encountered when adding new tryptamines to an existing confirmation panel, highlights different "mushroom" products, and relays which analytes were confirmed in blood and urine specimens.

Methods: The laboratory's previously published LC-MS/MS method for psilocin in blood and urine was modified to include 4-AcO-DMT, 4-acetoxy-*N*,*N*-diethyltryptamine (4-AcO-DET), 4-hydroxy-*N*,*N*-diethyltryptamine (4-OH-DET), 4-hydroxy-*N*-methyl-*N*-ethyltryptamine (4-OH-MET), 4-hydroxy-*N*,*N*-diisopropyltryptamine (4-OH-DiPT), and 4-hydroxy-*N*-methyl-*N*-isopropyltryptamine (4-OH-MiPT). The new analytes were qualitatively validated to ANSI/ASB Standard 036. Blood and urine specimens were screened by the laboratory's production LC-QTOF/MS method and confirmed by this expanded LC-MS/MS panel beginning in May 2025.

Results: The updated LC-MS/MS confirmation method was successfully validated; however, limitations and challenges were observed during development and validation. One of the main chromatographic limitations was the inability to resolve 4-OH-MiPT and 4-OH-DET isomers, despite assessing multiple analytical columns and gradients during development. Another analytical challenge was that the 4-AcO-DMT and 4-AcO-DET commercial standards inherently contained psilocin and 4-OH-DET, respectively. Furthermore, as these standards aged the concentration of the corresponding hydroxy analyte increased over time. Since May 2025, 42 cases were positive for psilocin, 16 were positive for 4-OH-MET, and 10 were positive for 4-OH-DET/4-OH-MiPT, with several cases confirming positive for multiple tryptamines. Of the 22 cases with synthetic tryptamines, only 4 contained psilocin.

Discussion: In 2024, descriptions of different types of products beyond mushrooms began appearing in case histories. Many of these products were depicted as mushroom chocolate bars and gummies infused with mushroom extracts. After the updated analytical method went live, 18 cases were confirmed positive for 4-OH-DET/4-OH-MiPT and/or 4-OH-MET, where no psilocin was detected. Several cases alluded to ingestion of mushroom

gummies or mushrooms in general, with only one instance where the submitter suspected use of “synthetic mushrooms”. These results emphasize how the abuse of hallucinogenic tryptamines is gaining momentum and expanding into synthetic analogs to evade detection and accountability. This validated analytical method addresses the emergence of psychedelics that may pose a risk to health, safety, and readiness.

Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

O-183

Qualitative Detection of MDMB-4en-PINACA and 5F-ADB Metabolites Using HPLC-DAD and GC-QQQ/MS

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Abstract

Introduction: High-resolution mass spectrometry (HRMS) is the current gold standard for detecting New Psychoactive Substance (NPS) metabolites. However, its high cost and operational complexity remain significant barriers for many forensic laboratories. Synthetic cannabinoids present unique challenges due to extensive metabolism, often leaving parent compounds undetectable in biological matrices. This study evaluates HPLC-DAD and GC-QQQ/MS as accessible, cost-effective alternatives for the qualitative identification of diagnostic metabolites of MDMB-4en-PINACA and 5F-ADB, whose gas chromatography parameters are currently unrepresented in current screening literature.

Objectives: The objective of this study is to evaluate the instrumental capability in the qualitative detection of the butanoic acid metabolites of MDMB-4en-PINACA and 5F-ADB using HPLC-DAD and GC-QQQ/MS. Furthermore, the study aims to assess the suitability of these more widely available techniques as practical alternatives to HRMS for routine forensic toxicology screening by cross-verifying workflow performance against an established HRMS baseline.

Methods: Urine samples from routine forensic casework were subjected to alkaline hydrolysis (200 µL of 11.8 M KOH added to 3 mL of urine, heated at 60°C for 15 min). After adjusting the pH to 2 with 1% HCl, analytes were extracted via liquid-liquid extraction using a 80:10:10 mixture of hexane, ethyl acetate, and diethyl ether. After vortexing and centrifugation, the top organic layer was evaporated to dryness.

For HPLC-DAD analysis, residues were reconstituted in 200 µL of acetonitrile and analyzed at a wavelength of 302 nm using an acetonitrile and 1% formic acid in water gradient. For GC-QQQ/MS analysis, samples were derivatized with 70 µL of BSTFA at 60°C for 15 minutes to facilitate the formation of trimethylsilyl (TMS) derivatives. Certified reference standards were utilized to establish retention times and multiple reaction monitoring (MRM) transitions. Method suitability for low casework-relevant concentrations was evaluated via matrix-matched verification using spiked blank human urine, with all analytical findings cross-verified for definitive identification via high-resolution LC-QTOF/MS. The primary transitions monitored for the TMS-derivatized metabolites were as follows: 5F-ADB metabolite 7 TMS [435 → 318 (mass ion); 318 → 145, 213, 233; 233 → 145] and MDMB-4en-PINACA butanoic acid TMS [415 → 213 (mass ion); 298 → 145, 171, 213].

Results: The methods successfully identified both metabolites in authentic urine samples (n = 50). In routine casework, results demonstrated high concordance between analytical platforms; HPLC-DAD detections were consistently confirmed by GC-QQQ/MS and vice versa, both of which were cross-confirmed by the established LC-QTOF/MS method. Matrix-spiking demonstrated robust selectivity with zero interferences and established a functional LOD of 1 ng/mL. Both platforms reliably detected these diagnostic markers in instances where parent compounds were completely consumed.

Discussion: While HRMS remains the preferred standard for unknown screening, this study demonstrates that GC-QQQ/MS using targeted MRM transitions provides sufficient selectivity and sensitivity for routine identification of synthetic cannabinoid metabolites. The use of specific parent-to-daughter ion transitions allowed for definitive qualitative detection even in complex urine matrices where parent compounds were completely consumed. HPLC-DAD at 302 nm served as an effective secondary screening tool, although GC-QQQ/MS provided superior selectivity for definitive forensic confirmation. This dual-platform approach offers a robust, cost-effective workflow for laboratories lacking HRMS instrumentation.

Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

O-184

Green Analytical Toxicology – Development of a Sustainable Qual-Quant Workflow Exemplified for Neuroleptics and Antidepressants in Human Plasma

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Abstract

Introduction: Monitoring antidepressants and neuroleptics, which are frequently involved in intoxications, is essential in analytical clinical toxicology. However, conventional liquid chromatography high-resolution tandem mass spectrometry (LC-HRMS/MS) workflows often require large volumes of solvents resulting in substantial waste and environmental burden. Consequently, there is a growing demand for miniaturized analytical workflows that maintain high sensitivity and selectivity while minimizing solvent waste in line with the principles of Green Analytical Chemistry (GAC).

Objectives: This study aimed to develop and validate a sustainable workflow to simultaneously identify and quantify 59 frequently prescribed antidepressants and neuroleptics in human plasma. The method used microextraction and subsequent microflow LC-HRMS/MS to minimize solvent waste while maintaining analytical performance.

Methods: Sample preparation was performed via miniaturized liquid-liquid extraction (micro-LLE). One hundred microliters of human plasma were spiked with a mixture of five isotope-labelled internal standards (citalopram-d_6, norclozapine-d_8, nordazepam-d_5, trimipramine-d_3, zolpidem-d_6) and extracted using 500 µL of ethyl acetate/diethyl ether mixture (1:1, v/v). After evaporation to dryness and reconstitution in 100-µL of methanol, 0.8 µL was injected onto the LC-HRMS/MS system. The analytes were separated using a ThermoFisher Scientific (TF) Hypersil GOLD C18 column (1.9 µm, 1 mm x 100 mm) and a flow rate of 100 µL/min over an 11.5 min gradient. The mobile phases consisted of aqueous ammonium formate and acetonitrile (both with 0.1% formic acid). The samples were analyzed using positive heated electrospray ionization in full scan mode on a TF Q Exactive Orbitrap, followed by data-dependent MS^2. The greenness of the analytical workflow was assessed using the Analytical GREEnness calculator (AGREE), Analytical Method Volume Intensity (AMVI) and Green Analytical Procedure Index (GAPI) metrics.

Results: All 59 analytes were successfully separated and detected with limits of detection (LOD) sufficiently low to cover clinically relevant toxicological concentration ranges. For most analytes, LODs were within the subtherapeutic concentration range, while for a minor number of low-dosed drugs, the LODs were still below plasma concentrations associated with toxic effects. The method was found to be selective for all analytes and no carry-over was observed. Calibration curves showed sufficient linearity over the investigated concentration ranges, with reproducible recovery and only minor matrix effects. Quantification was possible across relevant concentration ranges of the investigated drugs. The mobile phase consumption was decreased by nearly 80% compared to conventional flow rates. Greenness evaluation using AGREE, AMVI and GAPI tools emphasized the environmental sustainability

of the workflow, showing an 85% AMVI reduction compared to conventional methods and highlighting the reduced waste production.

Discussion: Combining micro-LLE and microflow LC-HRMS/MS represents a step toward "greener" clinical toxicology. The low extraction volume together with a reduced low flow rate substantially decreased the environmental footprint of the analytical workflow. This approach demonstrated that miniaturization strategies in LC-MS analysis can effectively support environmentally sustainable laboratory practices while still providing robust analytical performance. At the same time, this method enabled comprehensive multidrug screening, highlighting its potential as a practical tool for routine toxicological analysis.

Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

O-185

Switching It Up: A Novel Method Using Switchable Hydrophilicity Solvent Liquid-Liquid Microextraction and LC-MS/MS for the Analysis of Traditional and Novel Stimulants in Blood

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Abstract

Introduction: Synthetic cathinones pose a public health issue and have been reported in forensic casework. These substances are not always included in the scope of routine methods, requiring the development of novel workflows. Recently, the field of forensic toxicology is advancing towards implementing green analytical chemistry (GAC) principles. One proposed strategy is the use of switchable hydrophilicity solvents (SHS) in liquid-liquid microextraction (LLME). Using SHS instead of traditional solvents results in reduced solvent volumes, minimal chemical waste and no use of chlorinated solvents.

Objectives: The aim of this study was to develop and validate a SHS-LLME method using *N*,*N*-dimethylcyclohexylamine (DMCHA) for the analysis of amphetamine, methamphetamine, MDA, MDMA, cocaine, pentylone, *N*-propyl butylone, *N*-isopropyl butylone, *N*,*N*-dimethylpentylone, and mephedrone in blood using liquid chromatography-tandem mass spectrometry (LC-MS/MS).

Methods: Blood (200 µL) was submitted to protein precipitation using 600 µL of acetonitrile and centrifugation for 5 min, at 10,000 rpm. The supernatant was collected, spiked with 50 µL of 10% HCl in methanol, and evaporated under a nitrogen flow. Next, 550 µL of DMCHA:6 M HCl (1:1, v/v) and 400 µL of 6 M NaOH were added, vortexed, and allowed to rest for 2 minutes. The solution was centrifuged (5 min, 10,000 rpm), and the organic layer was collected, evaporated under nitrogen flow, and reconstituted in 100 µL of mobile phase at starting conditions. SHS-LLME conditions were selected upon optimization using central composite design and response surface methodology. Analyses were performed using LC-MS/MS using gradient elution, positive electrospray ionization, and multiple reaction monitoring mode. The method was validated following ASB Standard 036 using bovine blood (calibration model, accuracy, precision, dilution integrity, matrix and common drug interferences, limit of detection, matrix effects, recovery and carryover) and applied to the analysis of 16 authentic blood specimens previously tested and donated by an external reference laboratory.

Results: Recoveries between 42% and 135% were considered acceptable. Limit of detection was 1 ng/mL for all analytes, except *N*-propyl butylone (9 ng/mL). Significant matrix effects were observed at low and high concentrations ranging from -88.7% to -55.8% and -79.4 to -17.3%, respectively. Amphetamine, eutylone, and methamphetamine only met the criteria for qualitative analysis. All the other analytes met the criteria for quantification following a quadratic model with a 1/x weighting (5 – 500 ng/mL, except for *N*-propyl butylone (9 – 500 ng/mL)). Acceptable bias and precision (± 20%) were observed for all analytes. Dilution integrity was evaluated at 1:2 from 1000 ng/mL and were accurately quantitated. The method was successfully applied to the analysis of authentic blood specimens. The environmentally friendliness score (between 0 and 1) was 0.48 using Analytical GREEnness (AGREE) and 0.4 using AGREEprep.

Discussion: This method was successfully validated and applied to authentic specimens, using a microextraction with reduced waste generation. Limitations of this method include extraction solvent effects for eutylone and *N*-propyl butylone, and variations for methamphetamine and amphetamine not normalized by the internal standard. Additionally, inherent environmental hazards of DMCHA and LC-MS/MS utilization impacted the overall greenness.

Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

O-186

Identification of Cannabinoids Related to Delta-9-THC Through Derivatization and GC-MS Analysis

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Abstract

Introduction: The cannabis plant (Cannabis sativa L.) contains numerous psychoactive compounds known as cannabinoids, some of which differ in structure by the presence of a carboxylic acid group, whereas others differ only by the location of a double bond. Identification of these compounds can be challenging due to chemical transformations that can occur during analysis and subtle differences in chemical structure. Not all cannabinoids are controlled at the federal or state levels. Careful analysis of these compounds is particularly important to drug analysis labs which must differentiate between controlled and non-controlled substances to properly support the enforcement of drug laws.

Objectives: Five isomers of tetrahydrocannabinol in which the double bond was located at different positions in the A-ring and delta-9-tetrahydrocannabinolic acid-A were derivatized by two separate techniques and analyzed by gas chromatography-mass spectrometry (GC-MS).

Methods: DEA-exempt standard solutions of delta-9-THC, delta-9-THCA-A, delta-8-THC, delta-10 (6aR,9R)-THC, delta-6a,10a (9R)-THC, and exo-THC (aka. Delta-11-THC) were purchased from Restek at 1.0 mg/mL, transferred quantitatively and diluted to 10 mL with acetonitrile or chloroform to produce 0.1 mg/mL secondary stock solutions. Samples were derivatized with BSTFA containing 1% TMCS and MTBSTFA containing 1% TBDMCS (250 mcL of sample and 50 mcL of derivatizing reagent were heated at 70 C for 20 minutes). For standards that were received as methanol solutions, methanol was removed prior to derivatization by evaporating an aliquot to dryness under an air stream with mild heating, then the residue was redissolved in chloroform or acetonitrile. This evaporation was performed on the secondary stock solutions that contained a combination of methanol and acetonitrile or chloroform. All analyses were performed on an Agilent 5975C/7890A GC-MSD fitted with Rxi-5 Sil w/guard column (30 m x 0.25 mm ID x 0.25 mcm load).

Results: The five THC isomers in which the double bond was moved around the A-ring were differentiated using TMS and TBDMS derivatization. Additionally, Delta-9-THC was differentiated from delta-9-THCA-A using the same techniques. Analytes were identified using a combination of retention times and mass spectral data with strong signals characteristic of the two different derivatization groups (TMS and TBDMS).

Discussion: Our laboratory has established that employing a combination of two derivatization techniques provides a high degree of specificity in the identification of five THC isomers. We also found that delta-9-THC can be differentiated from delta-9-THCA-A using the same techniques. Future research will include analysis of other commercially available THC isomers such as delta-10 (6aR,9S)-THC (a diasteriomer of delta-10 (6aR,9R)-THC) and related compounds such as HHC. However, future work will not include the commercially available delta-6a,10a (9S)-THC that would not be expected to separate or give a unique mass spectrum given that it is the enantiomer of delta-6a,10a (9R)-THC. This work is important to drug analysis labs in support of court proceedings.

Disclosure

Yes, I, or a member of my immediate family, has a financial interest to disclose.

Conflict of Interest

A portion of this work was a collaboration with Restek Corporation. My conference registration will be paid by Restek.

O-187

Phenol in Postmortem Heart Blood: Produced by Decomposition or Exogenous Intake During Life?

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Abstract

Introduction: The source identification of phenol in post-mortem biological samples is a long-standing core challenge in forensic toxicology. Phenol can either originate from the deceased's ante-mortem exogenous exposure through dermal application, respiratory inhalation or gastrointestinal ingestion, or be an endogenous product naturally formed during corpse decomposition. The distinction between the two sources directly determines the accuracy of the case's cause-of-death conclusion. This study is based on an actual death case, focusing on the traceability of phenol in post-mortem cardiac blood to resolve the forensic identification dispute over the source of detected phenol.

Objectives: This study aims to resolve the forensic identification dispute over whether the detected phenol in post-mortem cardiac blood is endogenously produced by corpse decomposition or derived from ante-mortem exogenous exposure, and provide solid scientific evidence for the cause-of-death determination of this case and similar phenol-related cases.

Methods: Liquid-liquid extraction coupled with gas chromatography-mass spectrometry (GC-MS) was adopted for pre-treatment and precise qualitative and quantitative detection of three core biological specimens: post-mortem cardiac blood, liver tissue and gastric tissue. Auxiliary screening was performed using decomposition-related markers such as o-cresol. The toxicological distribution characteristics were cross-verified with case background information and post-mortem autopsy findings to establish a multi-dimensional phenol source identification system covering concentration, distribution and autopsy.

Results: The detected phenol levels in the deceased's cardiac blood, liver tissue and gastric tissue were 180 μg/mL, 0.45 μg and 1.11 μg/g respectively. No exclusive characteristic markers for phenol source tracing were detected. The cardiac blood showed a relatively high phenol concentration, while the liver did not exhibit the high accumulation feature corresponding to exogenous phenol poisoning, and no characteristic high-concentration phenol residue associated with acute oral phenol ingestion was detected in the gastric tissue. Autopsy comparison confirmed the absence of exogenous exposure features, and it was finally confirmed that all detected phenol was naturally generated during corpse decomposition.

Discussion: The core metabolites of phenol in vivo (hydroquinone, catechol, phenyl sulfate and phenyl glucuronide) can be generated not only by the metabolism of exogenously ingested phenol, but also converted from aromatic precursors produced by normal human metabolism or dietary intake, and no exclusive characteristic markers for source tracing are available, so the metabolites of phenol cannot be used as markers of exogenous intake. The multi-dimensional tracing method established in this study can efficiently distinguish the source of phenol in post-mortem biological samples. The obtained phenol concentration data enriches the dataset of phenol concentration distribution for decomposition-generated phenol.

The multi-dimensional cross-verification successively excludes common exogenous exposure routes, providing a replicable practical path for difficult forensic toxicology identification cases related to phenol, and effectively filling the identification gap of decomposition-generated phenol in such scenarios.

Disclosure

Yes, I, or a member of my immediate family, has a financial interest to disclose.

Conflict of Interest

This study was supported by the National Key Research and Development Program of China (Grant No. 2023YFC3303904).

O-188

Evaluating Strategies for Low-Level Quantification of THC Isomers and Metabolites in Whole Blood by Conventional GC-MS Instrumentation

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Abstract

Introduction: Liquid chromatography–tandem mass spectrometry (LC-MS/MS) is the preferred technique for drug and metabolite analysis in biological matrices due to its sensitivity, selectivity, and minimal derivatization requirements. However, analysts may need to develop methods using conventional gas chromatography–mass spectrometry (GC-MS) due to limited LC-MS/MS access and the widespread availability of GC-MS platforms.

Objectives: The objective of this work was to evaluate and mitigate the challenges of low-level drug analysis in biological matrices using conventional GC-MS through comprehensive method optimization. Analysis of THC isomers and metabolites in whole blood served as a representative test case due to the low detection limits required. Eleven analytes including 11-hydroxy-Δ8-tetrahydrocannabinol, 9(R)-hexahydrocannabinol, and (6aR,9R)-Δ10-tetrahydrocannabinabinol were analyzed.

Methods: Thermodynamic modeling software was employed to develop an optimized chromatographic separation on a 5-type column. A selected ion monitoring (SIM) method was established to optimize dwell times and leverage shared fragment ions between analytes. Following method development, a calibration curve was prepared in ethyl acetate to evaluate the sensitivity of a conventional GC-MS system (Agilent 7890B GC coupled with a 5977B MS, standard electron ionization source). Method performance was subsequently assessed in whole blood samples prepared via liquid–liquid extraction (LLE) and spiked post-extraction to evaluate sensitivity and selectivity in a complex matrix. Whole blood was acidified with 10% acetic acid then extracted with 9:1 hexane:ethyl acetate. Extracts were then spiked, dried, and derivatized with BSTFA + 1% TMCS.

Results: All analytes demonstrated acceptable sensitivity in solvent at a final extract concentration of 5 ng/mL, corresponding to 0.5 ng/mL in whole blood. Calibration curves exhibited acceptable linearity, with r^2 values greater than 0.9990 for all analytes except Δ9-THC (r^2 = 0.9982). Elevated back-calculated accuracies were observed for four analytes at the low calibration range. As expected, matrix interferences were significant for several monitored ions in whole blood. Acceptable sensitivity (S/N > 5) was achieved at 1 ng/mL in whole blood for at least one ion per analyte, with the exception of (6aR,9R)-Δ10-tetrahydrocannabinol and 11-nor-9-carboxy-Δ8-tetrahydrocannabinol, which showed substantial interference across all monitored ions. Although less sensitive than LC-MS/MS, this LOD is applicable to established working ranges for these analytes in whole blood. Triplicate injections of a post-extraction spiked sample (equivalent to 25 ng/mL in whole blood) yielded %RSD values of 1.0–10.6% after internal standard normalization.

Discussion: These results demonstrate that while conventional GC-MS systems cannot achieve the performance of advanced triple quadrupole platforms, they can support low-level quantification of cannabinoids and related metabolites in biological matrices when methods

are carefully optimized. Key factors influencing performance include chromatographic resolution for analytes as well as matrix interferences, mass spectrometric parameter optimization, and instrument maintenance. Increased variability at the low end of the calibration range highlights the need for further refinement to achieve robust quantification for all analytes. Ongoing work building on these results will be presented, exploring the use of thermodynamic modeling to better characterize and mitigate matrix interferences, as well as alternative derivatization strategies and detector optimization to enhance sensitivity.

Disclosure

Yes, I, or a member of my immediate family, has a financial interest to disclose.

Conflict of Interest

Salary

O-189

Identification and Chiral Separation of N-Ethylpentedrone (NEP) and its Metabolites by Capillary Electrophoresis-Mass Spectrometry Using Cyclodextrins as Chiral Selectors

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Abstract

Introduction: 2-(Ethylamino)-1-phenylpentan-1-one (N-ethylpentedrone, NEP) is a synthetic cathinone structurally related to methcathinone and pentedrone. Since its first identification on the European drug market in 2013, NEP has been monitored as a new psychoactive substance within the EU Early Warning System. In recent years, its relevance has increased due to a marked rise in the availability of synthetic cathinones across Europe, with large-scale imports and seizures reported between 2020 and 2024.

Objectives: This study aimed to develop and optimize both achiral and chiral CE-MS/MS methods for the analysis of N-ethylpentedrone (NEP) and its phase I and phase II metabolites. The achiral approach focused on achieving sensitive identification of NEP and its metabolites, meanwhile the chiral method was developed to enable enantiomeric separation using cyclodextrins as chiral selectors. Additionally, the study explored the detectability of major metabolites under different analytical conditions and assessed the applicability of the developed methods to biological matrices.

Methods: Analyses were performed by capillary electrophoresis coupled with tandem mass spectrometry (CE-MS/MS) using an unmodified fused-silica capillary (100 cm). Achiral separations were carried out using 20 mM ammonium formate (pH 6.5) as background electrolytes under positive and negative ionization mode. Chiral separations were achieved by partial capillary filling (80%) using sulfobutyl ether-β-cyclodextrin (SBE-β-CD) as chiral selector at 6 mg/mL concentration. To enhance sensitivity, pH-mediated sample stacking was applied by reconstituting the samples in 20 mM ammonium formate buffer at pH 2.3, while 20 mM ammonium formate buffer at pH 6.5 was used as the background electrolyte. This strategy promotes the preconcentration of analytes inside the capillary, representing one of the advantages of CE-MS/MS. NEP positive urine samples were then analyzed under the aforementioned methodologies.

Results: Since none of the investigated metabolites were detected in negative ionization mode, both achiral and chiral methods were developed using positive ionization mode. The developed achiral CE-MS/MS method enabled the detection of NEP and several phase I metabolites in different urine samples, with good signal intensity, including nor-NEP, hydroxy-NEP, dihydro-NEP, and carboxy-NEP. In addition, phase II metabolites, such as hydroxy-NEP glucuronide and a putative diglucuronide, were identified under optimized conditions. Chiral separations using SBE-β-CD allowed baseline enantiomeric resolution of NEP and selected metabolites. Sensitivity was

significantly improved by applying pH-mediated stacking strategies compared to non-stacking conditions, particularly in achiral mode.

Discussion: The proposed CE-MS/MS approach demonstrated the capability to detect NEP and its major phase I and some phase II metabolites in urine samples, highlighting its applicability for toxicological analysis. The use of cyclodextrins in a partial filling configuration enabled enantioselective interactions, providing chiral discrimination for NEP and selected metabolites in real biological samples. The obtained baseline enantiomeric separation underlines the potential of CE-based methods for chiral analysis of synthetic cathinones. Overall, the method represents a promising analytical strategy that could be further optimized to improve robustness and enantiomeric resolution in other complex biological matrices.

Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

O-190

Chiral Separation and Identification of Ketamine, Norketamine, and Medetomidine: Method Development and Application to Forensic Samples

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Abstract

Introduction: Forensic toxicologists face the challenges of not only identifying new drugs but also distinguishing enantiomeric forms of certain drugs. A chiral drug is has the same molecular formula and structural composition, but the spatial arrangement is different, causing different pharmacological effects. Knowing which enantiomer is present in an individual can help determine the source of the drug. The S-conformation of ketamine (esketamine®) has been approved for treatment of refractory depression, whereas illicit ketamine is traditionally a racemic mixture and the enantiomers are metabolized at different rates. Medetomidine is traditionally used in veterinary medicine but has infiltrated the drug market in its racemic form as an adulterant.

Objectives: This study focuses on development and validation of a new analytical method for the chromatographic resolution of: (R)- and (S)-ketamine, (R)- and (S)-norketamine, dextro- and levo-medetomidine. Liquid chromatography–tandem mass spectrometry (LC-QQQ-MS) was employed to separate the enantiomeric pairs.

Methods: Analytical standards for each enantiomer and corresponding internal standards were purchased and infused independently. Chromatographic separation was achieved using a Phenomenex Lux Cellulose-3 (150x2.0 mm 3.0 um) chiral analytical column with an isocratic gradient on an Agilent 1290 Ultra High-Performance Liquid Chromatograph coupled to the Agilent 6495 Triple Quadrupole Mass Spectrometer. The method, which is currently being validated qualitatively, was adapted from *Walton et al. 2025* in the Journal of Analytical Toxicology. Mobile phase compositions are 20 mM ammonium formate and 0.01% ammonium hydroxide in water (A) and methanol (B). The injection volume is 10 µL with a column temperature of 35ºC and a flow rate of 0.3 mL/min, and a total run time of 14 minutes. The following gradient was used: 55% B with an 8-minute hold, ramping to 90% B for 2.9 minutes, then ramping to an organic flush at 95% B for 0.9 minutes, and returning to initial conditions at 12.10 minutes with a re-equilibration time of 1.9 minutes. The instrument is operated in ESI+ with MRM.

Results: 15 cases containing medetomidine, ketamine, or norketamine, from 10 US states between April 2025 and December 2025, in addition to two oral fluid samples in 2025, were collected and analyzed. Based on a LC-QTOF-MS qualitative screening results, ketamine, norketamine, or medetomidine, were detected alongside other traditional and NPS drugs. Five cases and both oral fluid samples that were qualitatively positive for ketamine were positive for both (R) and (S) – ketamine on the chiral assay. Four of those cases and both oral fluid samples were also positive for (R) and (S) – norketamine. Eight cases were positive for both dextro- and levo-medetomidine. Additionally, two drug chemistry samples that were submitted to the CFSRE and determined to contain ketamine and medetomidine, were analyzed using the chiral method. (R) and (S) – ketamine was identified in the ketamine sample, while both dextro- and levo-medetomidine was identified in the medetomidine sample.

Discussion: The method was successful in identifying and resolving (R)- and (S)-ketamine, (R)- and (S)-norketamine, dextro- and levo-medetomidine from authentic samples. As these drugs continue to be prevalent it is toxicologically relevant to be able to distinguish which enantiomer is present in forensically relevant samples.

Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

O-191

Z/E-Methoxime Isomers in Gas Chromatography-Quadrupole Mass Spectrometry Analysis of C6-keto-Opioids in Human Urine as Their Methoxime- and Acyl-Derivatives

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Abstract

Introduction: Hydrocodone, hydromorphone, oxycodone, and oxymorphone represent a group of C6-keto-opioids. Previously, it was discovered that the C6-C7 keto-enol tautomerization often occurred during sample derivatization for GC-MS analysis. One solution is to convert the C6 ketone to its corresponding methoxime-derivative followed by acylation or silylation. However, the procedure created an unexpected issue of generating *Z/E*-methoxime-derivative isomers that were usually distinct on the GC-MS chromatogram but difficult to be completely resolved. Thus, we initiated a study on formation and potential isomerization of these *Z/E*-methoxime-derivatives of C6-keto-opioids as part of our efforts to improve our GC-MS analytical method.

Objectives: To present analysis of the *Z/E*-methoxime derivatives of C6-keto-opioids and their isomerization under the non-aqueous Brønsted-Lowry acidic conditions in urine drug testing (UDT) by utilizing thermo- and kinetic studies and qualitative organic conformational analyses.

Methods: Our GC-MS method has been certified by the DoD and the National Laboratory Certification Program. Briefly, urine specimen with the internal standard was treated with concentrated HCl. After hydrolysis, the C6-keto-opioids in the specimen were converted to their corresponding methoxime derivatives. The mixture was then purified with an SPE and the eluate was dried under compressed air. The residue was heated with acid anhydride to convert the free hydroxy groups of the opioids to their esters. The resulting mixture was evaporated to dryness and reconstituted with ethyl acetate prior to GC-MS analysis.

Results: The peak from hydrocodone minor methoxime-derivative isomer consistently eluted approximately 6 seconds ahead of the major isomer. The minor peak existed in every monitored fingerprint ion window under the SIM mode and the minor isomer exhibited a mass fragmentation pattern that highly resembled to that of the major isomer under the Full Scan mode (Figure 1). Nearly identical phenomena were observed with hydrocodone-d_6.

The percent peak area of the minor isomer relative to the combined peak area of the two isomers consistently decreased with increased hydrocodone concentration (≈31%, 23%, 17%, 12%, 8%, 6%, 5%, 3%, and 2% corresponding to the hydrocodone concentrations of 10, 20, 40, 80, 100, 125, 150, 500, and 1000 ng/mL, respectively). In addition, lower temperatures during the methoxime conversion facilitated the increased relative peak area of the major isomer, which indicated that the major isomer was more kinetically favored.

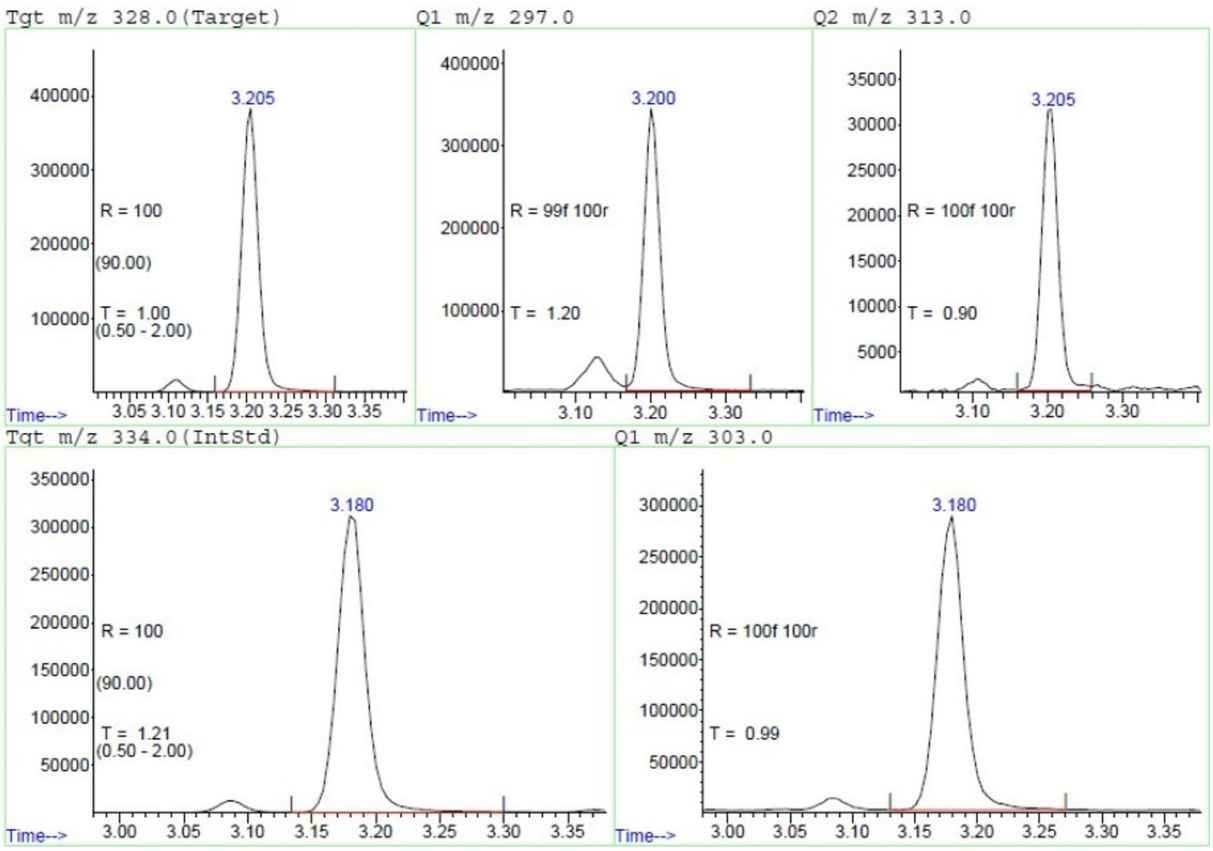
Once the methoxime was formed, it displayed excellent stereochemistry stability in gaseous phase. However, the two isomers are likely able to undergo an *E/Z*-isomerization under strong non-aqueous Brønsted-Lowry acidic condition with heat. With the same incubation time, elevated reaction temperatures also accelerated the isomerization. Similar mass spectral

phenomena were observed with hydromorphone, oxycodone, oxymorphone, and their corresponding deuterated internal standards.

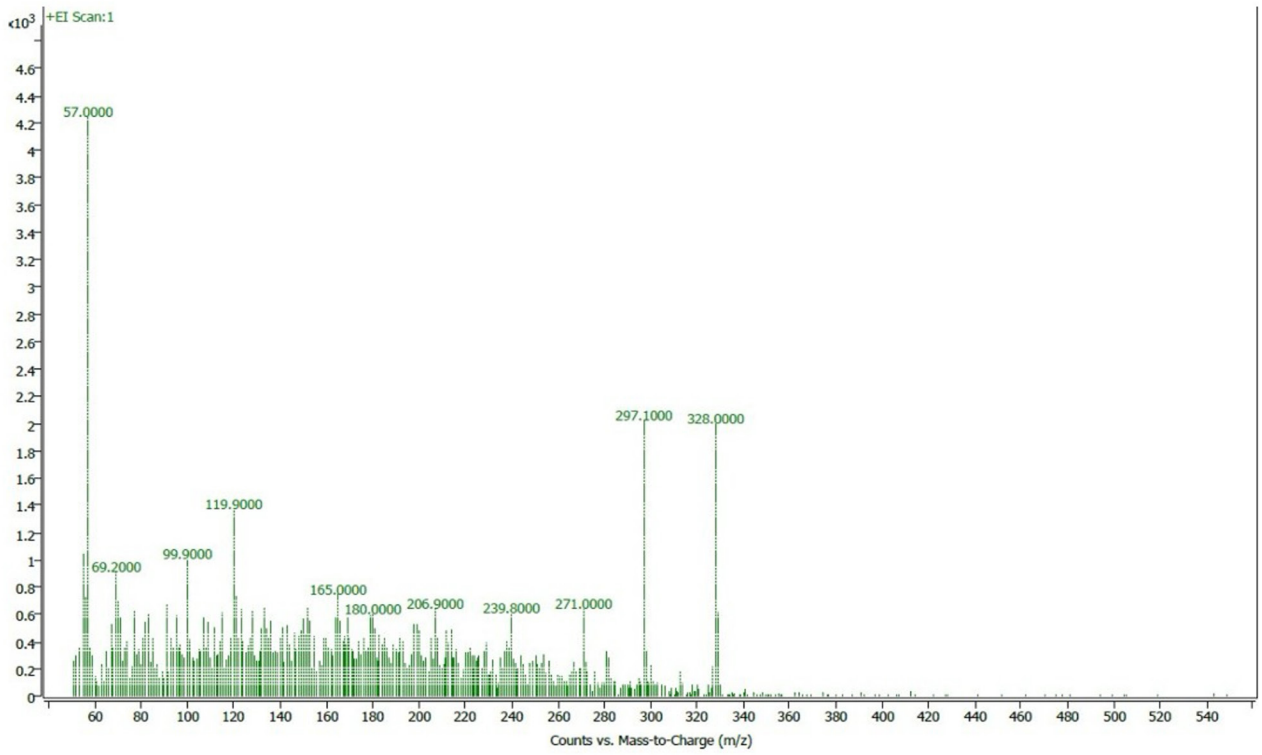
Discussion: We present an in-depth report and analysis of the *Z*/*E*-methoxime derivatives of C6-keto-opioids and their isomerization under the non-aqueous Brønsted-Lowry acidic conditions in UDT. Our findings and analyses offer valuable insights into these isomers and provided general guidance on optimization of the derivatization procedures.

Figure 1

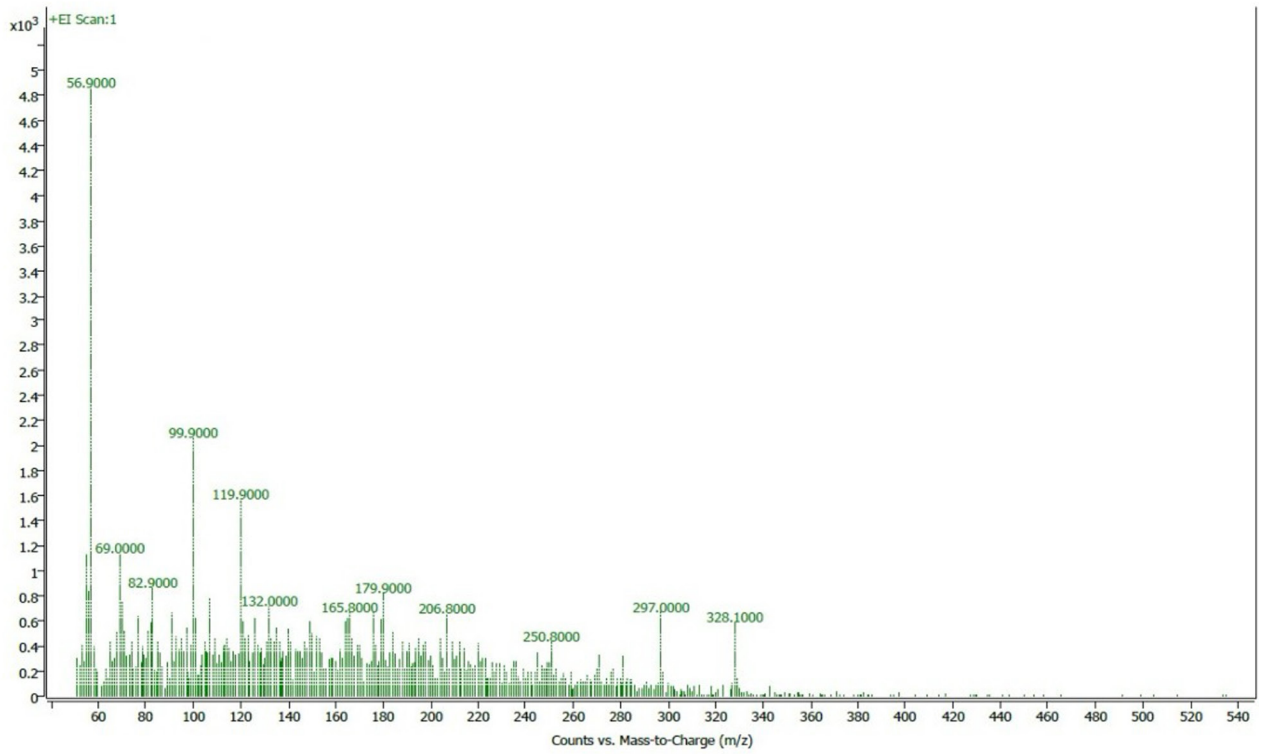
(a)



(b)



(c)



Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

O-192

Performance Comparison of LDTD-Coupled Orbitrap and Triple Quadrupole Mass Spectrometry for Urinary Screening of 23 Drugs Using Unified Extraction Method

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Abstract

Introduction: Mass spectrometry is one of the most powerful tools in toxicological analysis for examining biological matrices such as urine. Two main approaches can be distinguished: triple quadrupole mass spectrometers, primarily used for targeted analysis, and high-resolution accurate-mass systems such as Orbitrap instruments, which support both targeted and non-targeted investigations. Each approach presents distinct advantages and limitations.

Objectives: This study evaluated the performance of two mass spectrometry technologies coupled with laser diode thermal desorption for the urinalysis of 23 drugs. The platforms were compared for high-throughput urine drug panel analysis, with emphasis on their respective strengths and limitations.

Methods: For sample preparation, 50 µL of urine was spiked with internal standard and incubated with B-One enzyme and water for glucuronide deconjugation. A salt-assisted liquid–liquid extraction was then performed using acetonitrile and a NaCl/K_2HPO_4 buffer, followed by centrifugation. The supernatant was mixed with a desorption solution, and 6 µL was spotted onto a Lazwell96 plate, dried, and analyzed by LDTD-MS/MS (Phytronix). For exact mass analysis, an Exploris 240 (Orbitrap, Thermo Scientific) was used, while unit mass analysis was performed on a TSQ Altis Plus (Triple Quadrupole, Thermo Scientific). The LDTD parameters were identical for both systems. In negative ion mode, the laser ramped to 65% over 6 s, held for 2 s, then dropped to 0%. In positive ion mode, the laser increased to 85%, held for 2 s, and then returned to 0%. Gas flow was set at 6 L/min for both methods.

Results: Calibrators prepared in drug-free urine at 1X, 2X, and 5X cut-offs were used to compare exact-mass and triple quadrupole (unit-mass) detection. Signal responses were normalized to internal standards, and precision, accuracy, and interference were evaluated using triplicate analyses. Linearity was assessed from calibration curves, while classification performance was determined using quality control samples spanning 0.5X to 2X cut-offs. Exact-mass detection showed higher interference at the cutoff concentration for 86% of the drugs of abuse compared with triple quadrupole detection. Despite this, both platforms demonstrated excellent analytical performance, with R^2 values >0.99 for all analytes. Kappa scores exceeded 0.9012 for exact-mass detection and reached 1.0000 for triple quadrupole detection, indicating near-perfect agreement with the expected classifications.

Discussion: Triple quadrupole systems are designed for targeted analyses and remain the gold standard for achieving the highest sensitivity and lowest limits of quantification. In contrast, Orbitrap instruments offer greater versatility, supporting both targeted and non-targeted workflows with full-scan acquisition and additional scan modes, often combined with spectral libraries for compound identification. High-resolution accurate-mass instruments are particularly valuable in forensic contexts, as they enable retrospective data analysis to identify newly emerging synthetic

drugs in legal or post-mortem cases. However, achieving comparable quantification limits in non-targeted approaches generally requires longer acquisition times, reducing throughput. For routine applications, such as pre-employment testing where very low detection limits are required, unit mass analysis remains especially well suited.

Disclosure

Yes, I, or a member of my immediate family, has a financial interest to disclose.

Conflict of Interest

Salary/Consultant

L-001

From Fragments to Framework: Pre- and Post-Implementation Analysis of CLIO, an In-House Laboratory Information Management System

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Abstract

Introduction: Forensic toxicology laboratories rely on interconnected operational systems to manage casework from intake through final reporting. Historically, the San Francisco Office of the Chief Medical Examiner (OCME) managed these operations through a combination of commercial software, Microsoft Access, Microsoft Excel, and paper-based records. While leveraged as much as possible, these fragmented systems introduced inefficiencies, data gaps, and redundant manual entry across multiple platforms. In 2025, the OCME implemented CLIO, a fully in-house developed Laboratory Information Management System, customized designed to consolidate and automate laboratory operations across all domains.

Objectives: This study presents a pre- and post- implementation analysis of forensic laboratory operations following the implementation of CLIO. Operational domains analyzed include Cases, Specimens, Tests, Batches, Results, Reports, Records, Inventory, and Miscellaneous operations (Quality Assurance, Court Work, and Communications). Each domain was evaluated across five dimensions: (1) data consolidation — additional data points captured per operation; (2) time savings — staff time recovered per workflow; (3) duplication elimination — instances of redundant manual entry removed; (4) process expansion — new workflows enabled by automation; and (5) resource impact — effects on paper, physical storage, and staff effort.

Methods: CLIO was developed using a Microsoft SQL Server backend, a Python and Flask framework, and an HTML/JavaScript frontend, served on the City and County of San Francisco intranet. Development is performed by an in-house team with backgrounds in forensic toxicology, computer science, and bioinformatics. Pre-CLIO operational baselines were established by reviewing legacy systems including the commercial software, ToxDB (an internal legacy Microsoft Access database), paper logbooks, and Excel workbooks. Post-CLIO metrics were captured directly from CLIO's data models and workflow logs. Comparisons were made across all nine operational domains using the five analytical dimensions described above.

Results: Preliminary results demonstrate measurable improvements across all five analytical dimensions. Regarding duplication elimination, manual data entry redundancy was reduced by an estimated 66% across cases, specimens, and records, consolidating what was previously triplicate entry across FA, ToxDB, and paper systems into a single CLIO entry point. Regarding resource impact, the transition to CLIO eliminated a significant volume of paper-based recordkeeping, with at least 300 lbs of physical records replaced by digital entries since implementation in 2025. Quantitative analysis of data consolidation across all nine domains and time savings per workflow is currently underway, with findings to be presented in full at the time of the conference. Additionally, automation has enabled new workflows not previously possible, including two-person storage audits, direct specimen collection guidance to autopsy staff, QR-coded laboratory surface tracking, role-specific dashboards, and same-day system updates.

Discussion: The implementation of CLIO represents a fundamental shift in how the OCME manages forensic laboratory operations, moving from a fragmented, paper-heavy model to a unified, data-rich, and scalable automated system. Early analysis across five operational dimensions confirms substantial gains in efficiency, data quality, and process capability. By investing in in-house development, the OCME has built a sustainable platform whose value compounds as casework volume and operational scope grow.

Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

L-002

Evaluation of in Silico ADME and Toxicity Predictions for Novel Psychoactive Substances Using Percepta

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Abstract

Introduction: The rapid emergence of novel psychoactive substances (NPS) presents ongoing challenges for forensic/clinical toxicology, particularly due to limited experimental pharmacokinetic and toxicological data. *In silico* tools offer a rapid approach to estimate absorption, distribution, metabolism, excretion and toxicity (ADME-Tox) properties.

Objectives: To evaluate the performance of *in silico* ADME-Tox predictions by benchmarking against well-characterized traditional drugs, and to explore class-based patterns among 31 NPS.

Methods: A dataset of 31 NPS spanning six classes: synthetic opioids (n=12), synthetic cathinones (n=8), synthetic cannabinoids (n=2), novel benzodiazepines (n=3), dissociatives (n=3), and hallucinogens (n=3) was analyzed using Percepta (v14.53). Predicted endpoints included lipophilicity (LogP), Caco-2 and jejunal permeability (Pe), human intestinal absorption (HIA), plasma protein binding (PPB), volume of distribution (V*d*), CNS penetration (LogBB), CYP-mediated metabolic stability, organ-level toxicity probabilities, Ames mutagenicity, and skin/eye irritation. Absorption predictions were generated using both GALAS (similarity-corrected) and Classic (fragment-based) models. Traditional drugs (e.g., amphetamine, cocaine, morphine, benzodiazepines, and Δ^9-tetrahydrocannabinol (THC)) were used as benchmarks via a scoring scale (1–5), integrating agreement across predicted endpoints, where 5 represents strong agreement with known pharmacokinetic behavior.

Results: Predictions for traditional drugs showed strong agreement. THC scored highest (5), with a predicted LogP of ~7, consistent with its lipophilicity, distribution, and CNS penetration. Cocaine, amphetamine, morphine, and benzodiazepines scored 4–5, residual gaps reflect transporter or enzyme processes outside passive models. Across the 31 NPS, absorption was uniformly high (mean jejunal Pe 7.3 $\times 10^{-4}$ cm/s; maximum passive absorption ≈100%; transcellular contribution 99–100%; predicted oral bioavailability 86–99.9%), and GALAS/Classic outputs were highly concordant. Distribution and toxicity endpoints were class-discriminating. Synthetic opioids exhibited the highest PPB (mean 94%; 87–96%), the largest Vd (mean 16 L/kg; up to 35 L/kg), the strongest CNS penetration (mean LogBB 0.78), and elevated organ-effect probabilities for the lungs (~0.95) and cardiovascular system (~0.90), consistent with the respiratory-depression risk profile of opioids. Synthetic cathinones showed comparably high absorption but markedly variable PPB (44–90%), implying potentially elevated free fractions. Novel benzodiazepines presented compact distribution (V*d* 2–4 L/kg, PPB ~90%) and the lowest predicted toxicity probabilities. Dissociatives showed low PPB (27–50%), indicating weaker plasma retention. Classical hallucinogens were the most heterogeneous (Pe 2.1–7.1; PPB 49–95%; LogBB −0.25 to 0.67) and carried the highest skin/eye irritation probabilities. Synthetic cannabinoids exhibited extensive PPB (~87%) and CNS penetration; predicted Vd (2.5-7.7 L/kg) was consistent with their moderate lipophilicity and was comparable to THC (~3.4 L/kg), with no experimental Vd reported in humans., but predicted V*d* was lower than expected for the class, suggesting limitations in capturing extensive tissue partitioning.

Discussion: Benchmarking against traditional drugs demonstrated that *in silico* predictions reliably reproduce key physicochemical and absorption trends, particularly for lipophilicity and permeability, with strong GALAS/Classic agreement supporting the robustness of these endpoints. Class-level patterns in distribution and organ-toxicity probabilities tracked plausibly with known pharmacology. While these predictions are not the most accurate or a perfect substitute for empirical data, limited by undefined metabolic stability and low reliability for some endpoints, they can serve as a preliminary tool to guide initial understanding of emerging NPS.

Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

L-003

Time-Dependent Distribution in Tissue, Blood and Urinary of Three Emerging Ketamine Analogues: 2-MDCK, 2-FXE, and 2-oxo-PCE in Rodent Models

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Abstract

Introduction: Ketamine analogues have emerged as new psychoactive substances with increasing forensic and toxicological concern. Among them, 2-MDCK, 2-FXE, and 2-oxo-PCE are structurally related dissociative compounds, determining their time-dependent concentrations in biological matrices is essential for interpreting toxicological findings and estimating exposure timing. This study aimed to establish and apply a validated UHPLC-TQMS method to investigate the dynamic distribution of them in blood, urine, and major tissues in rodent models.

Objectives: This study aimed to characterize the time-dependent distribution of 2-MDCK, 2-FXE, and 2-oxo-PCE in major tissues, blood, and urine after single-dose administration within 24 h, and to provide experimental evidence for forensic specimen selection and toxicological interpretation.

Methods: KM mice received a mixed solution of 2-MDCK, 2-FXE, and 2-oxo-PCE by intraperitoneal injection at 5 mg/kg, and heart, liver, spleen, lung, kidney, brain, skeletal muscle, and blood samples were collected at 0.25, 0.5, 1, 2, 4, 8, 12, and 24 h. Urine was collected from Sprague Dawley rats placed after mixed administration at 1.5 mg/kg, within 12 h. Blood and urine samples were extracted twice with ethyl acetate, followed by vortexing, ultrasonic extraction, centrifugation, nitrogen evaporation, and reconstitution in 0.1% formic acid-water. Tissue samples were processed similarly after homogenization and membrane filtration before being analyzed by the established UHPLC-TQMS method.

Results: Tissue concentrations peaked at the first sampling point, indicating rapid absorption and distribution. At 15 min, the highest concentrations were generally observed in spleen, liver, and kidney, whereas heart and lung showed lower relative levels. Tissue concentrations declined sharply within 0.25–0.5 h and continued to decrease more slowly thereafter. After 4 h, concentrations in most tissues were below 10 ng/g, except for relatively slower clearance in liver. At 24 h, residual concentrations were mainly retained in spleen and heart. For 2-MDCK, approximate concentration ranges were 2.54–238.22 ng/g in heart, 1.53–985.70 ng/g in liver, 2.31–1300 ng/g in spleen, 2.24–172.71 ng/g in lung, 1.27–1015.65 ng/g in kidney, 1.74–575.60 ng/g in brain, and 1.07–493.07 ng/g in muscle. For 2-FXE, the corresponding ranges were approximately 3.30–146.03, 1.69–973.24, 1.80–903.33, 1.19–122.97, 1.52–932.44, 0.97–424.09, and 1.85–448.80 ng/g. For 2-oxo-PCE, concentrations were higher overall, ranging approximately 2.35–307.92, 0.85–1388.60, 2.08–1846.94, 0.89–285.42, 0.99–1035.43, 0.84–704.10, and 0.93–482.48 ng/g, respectively. In blood, all three substances decreased rapidly within the first hour and approached low, stable concentrations after 2 h (ranging from 2 to 0.2 ng/mL). Urinary data showed rapid excretion within the first 2 h, followed by a marked decline and gradual stabilization after 7 h.

Discussion: The three ketamine analogues showed rapid systemic distribution and elimination, with early enrichment in highly perfused or elimination-related organs and detectable residues persisting for at least 24 h. For forensic practice, liver and kidney may be useful matrices shortly after exposure, whereas spleen and heart may provide better detection potential at later post-exposure intervals. Overall, this study provides preliminary in vivo toxicokinetic evidence for 2-MDCK, 2-FXE, and 2-oxo-PCE and supports more informed biological specimen selection in forensic toxicological investigations.

Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

Conflict of Interest

This research was supported by the Key R&D Project of Sichuan Provincial Science and Technology Program (No. 2023YFS0418) and the National Natural Science Foundation of China (No. 82072111).

L-004

Increasing Carfentanil Positivity in Clinical Urine Specimens from 2024 to 2025

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Abstract

Introduction: Carfentanil is a powerful synthetic opioid developed for veterinary use as a tranquilizer for large animals, but is also used illicitly for its higher potency compared to fentanyl. Carfentanil is 10,000 times stronger than morphine and 100 times stronger than fentanyl. It is currently listed as a Schedule II substance by the DEA in the United States (US). In 2017, China listed it as a controlled substance, after which a decrease in positivity in the US was observed. However, since 2024, there has been a marked increase in positivity in clinical specimens across the US.

Objectives: This study is a retrospective review of the change in positivity for carfentanil and its metabolite norcarfentanil in clinical urine specimens from across the United States that were tested for novel psychoactive substances (NPS) at Quest Diagnostics between 2024 and 2025.

Methods: A retrospective analysis was done of clinical urine specimens submitted for NPS testing. This method includes NPS from 6 drug classes (designer fentanyl analogs, designer benzodiazepines, designer opioids, designer stimulants, synthetic cannabinoids, and other NPS substances). Carfentanil and its metabolite norcarfentanil are both in the designer fentanyl analogs class. A linear calibration curve was used, and cutoffs for carfentanil and norcarfentanil were 2 ng/mL and 10 ng/mL, respectively. Extraction consisted of hydrolysis, filtration, and dilution, followed by analysis on a SCIEX 6500+ LC/MS/MS system. Results were assessed to determine carfentanil and norcarfentanil positivity for 2024 and 2025. Poly-drug positivity with other NPS and other traditional drugs of abuse were also evaluated.

Results: In 2025, there were 12,118 specimens positive for at least 1 NPS. Carfentanil and/or norcarfentanil were present in 470 (3.9%) of these positive specimens. For comparison, in 2024, 0.5% of positive specimens were positive for carfentanil and/or norcarfentanil (39 of 8,384 positive specimens). Of the 470 specimens positive in 2025, 50 (10.6% of carfentanil/norcarfentanil positives) were positive for just the fentanyl analog class, 298 (63.4%) were positive for two NPS classes, 89 (18.9%) were positive for three NPS classes, and 31 (6.6%) were positive for four NPS classes. The most frequently co-positive NPS with carfentanil and/or norcarfentanil was xylazine (404 specimens or 85.9%), followed by acetyl fentanyl (233 specimens, 49.6%), and medetomidine (103 specimens, 21.9%). The most frequently co-positive non-NPS were methadone/EDDP (270 specimens, 57.4%), the cocaine metabolite benzoylecgonine (210 specimens, 44.7%), and methamphetamine (172 specimens, 36.6%). Fentanyl and/or norfentanyl were detected in all positive specimens containing carfentanil and/or norcarfentanil.

Discussion: When comparing positivity between 2024 and 2025, there was an increase in carfentanil/norcarfentanil presence in positive NPS specimens from 0.5% to 3.9%. This is vital information for physicians and researchers because even though there may be a decrease in positivity for a drug at present, there is always the possibility of re-emergence. Keeping up to date on drug trends is important for research as well as for developing effective treatment for patients.

Disclosure

Yes, I, or a member of my immediate family, has a financial interest to disclose.

Conflict of Interest

Salary

L-005

A Methadone and Benzodiazepine Nal-Von-Minden Screening Test Could Not Be Confirmed: A Belgian NPS Puzzle of 4 Lethal Cases Involving Methiodone

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Abstract

Introduction: In February 2026, samples from a deceased, known user of recreational drugs, were analysed for elucidation of the cause of death. Samples from a full autopsy were available (including blood, urine, vitreous humour and stomach content), along with a white substance sampled from the nose of the deceased. A Nal-Von-Minden urine screening test was positive for methadone, benzodiazepines, amphetamine and methamphetamine.

Methods: Blood, urine and vitreous humour samples were first subjected to comprehensive drug screening by combining liquid chromatography with diode array detection (LC-DAD) and LC coupled to high-resolution mass spectrometry (LC-HRMS) measurements. For further elucidation, stomach content and the white substance sampled from the nose were analysed with both techniques as well. Gas chromatography-mass spectrometry was performed for additional confirmation. For identification of unknown compounds, open source libraries such as HighResNPS were used. Missing reference standards were obtained from LGC or Cayman Chemicals. Retrospective data-analysis was performed on all forensic cases from January 2025 on.

Results: Upon first analytical screening of blood, urine and vitreous humour, the positive result for methadone, as indicated by the immunoassay screening, could not be confirmed, neither with LC-DAD, nor with LC-HRMS: neither methadone nor its metabolite EDDP were present. Moreover, while, based on spectral match and retention time, the LC-DAD indicated the presence of diazepam, no benzodiazepine from our standard in-house screening library could be identified via LC-HRMS. Using open-source libraries and the HighResNPS library to evaluate our LC-HRMS data, phenazolam (clobromazolam; mono-isotopic mass 385.9934), desalkylgidazepam (bromonordiazepam; mono-isotopic mass 314.0055), phenibut (mono-isotopic mass 179.0946) and clobutinol (mono-isotopic mass 255.1623) were tentatively identified (i.e. based on spectral match, but not with the standards at hand). However, a major peak (mono-isotopic mass 345.1763), present in the substance sampled from the nose, could not be identified. Based on the initial methadone-positive screening result and being aware that international colleagues had seen methiodone in some cases, methiodone was tentatively identified based on its m/z. Sourcing of the reference standards allowed confirmation of the presence of phenazolam, desalkylgidazepam phenibut and methiodone ($C_{20}H_{27}NO_2S$), but not clobutinol. While elucidating the unknowns in this specific case, end of March and early May 2026 additional methiodone positive fatal cases were confirmed, in which the substance had been snorted as well. Moreover, retrospective data analysis revealed a fourth case dating back to August 2025. These four cases share compounds with mono-isotopic mass (predicted formulas) of 331.1606 ($C_{19}H_{25}NO_2S$) 317.1449 ($C_{18}H_{23}NO_2S$), 269.1196 ($C_{18}H_{23}NO$) and 255.1623 ($C_{17}H_{21}NO$), which aren't present in other forensic samples. While the first two correspond to methiodone dealkylation metabolites, $C_{18}H_{23}NO$ is an impurity found in the seized samples accompanying the cases, with 255.1623 corresponding to a dealkylation metabolite of this impurity.

Discussion: The analytical findings in these cases point to the importance of not relying on one sole analytical technique upon confirming screening results, as well as on the importance of international collaboration (by contributing to open source libraries) upon first detection of NPS in forensic cases. For this reason, we contributed the methiodone HRMS spectrum to the HighResNPS library.

Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

L-006

When Standards Don't Exist, the Samples Do: Navigating Diastereomeric Complexity in Mitragynoid LC-MS/MS Analysis

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Abstract

Introduction: The mitragynoid drug market now includes semi-synthetic alkaloids such as 7-hydroxymitragynine, mitragynine pseudoindoxyl, and dihydro-7-hydroxymitragynine, with the former two also being well-characterized products of mitragynine's major metabolic pathway. Mitragynine's three chiral centers yield eight possible diastereomers, four of which are routinely encountered in standard mitragynine casework. Due to structural similarity, it is hypothesized that the metabolic pathways of speciogynine, mitraciliatine, and speciociliatine may mirror that of mitragynine, producing corresponding 7-hydroxy and pseudoindoxyl metabolites. Without adequate chromatographic resolution of these diastereomers, misidentification or overreporting becomes an analytical risk.

Objectives: To develop an LC-MS/MS gradient method that resolves 7-hydroxymitragynine and mitragynine pseudoindoxyl from their speciogynine, mitraciliatine, and speciociliatine diastereomeric counterparts, leveraging authentic post-mortem samples to anchor retention time assignments for where commercially available standards are unavailable.

Methods: Development efforts were conducted on a Thermo Scientific™ Vanquish™ Flex LC System paired with a Thermo Scientific™ TSQ Quantis™ triple quadrupole tandem mass spectrometer. A Phenomenex Kinetex 2.6 µm phenyl-hexyl 100 Å, 100 x 2.1 mm column coupled with 0.1% formic acid in methanol and 0.1% formic acid in water served as the basis of the gradient method. Mobile-phase gradient development was initially guided by certified reference materials (CRMs) commercially available at the time of this study, then subsequently refined using authentic samples with high-abundance signals for mitragynine, speciogynine, speciociliatine, and mitraciliatine.

Results: As a primary reporting target, 7-hydroxymitragynine required isolation from all coeluting interferences. This was achieved by isolating it first from 7-hydroxyspeciogynine using a commercially available CRM, then from a separate unknown peak present in all the authentic samples analyzed. The identity of the unknown peak was not an analytical objective, though it is hypothesized to represent one or both of the remaining 7-hydroxy diastereomers: 7-hydroxyspeciociliatine and 7-hydroxymitraciliatine.

Unique transition ions were deliberately selected for the pseudoindoxyl diastereomers to ensure confident peak assignment as they are structural isomers of the 7-hydroxy compounds. Chromatographic peaks corresponding to speciogynine, speciociliatine, and mitraciliatine pseudoindoxyl were not observed in authentic sample extracts, initially suggesting complete coelution of all four diastereomers. Subsequent literature review identified a potential explanation: Angyal et al.[1] describe a dynamic diastereomeric equilibrium of the pseudoindoxyl scaffold in protic environments, which may preclude discrete chromatographic resolution regardless of method conditions; this reframes the absence of distinct peaks as a chemical property of the pseudoindoxyl scaffold rather than a method limitation.

Discussion: The growing prevalence of mitragynoids in the drug supply demands that analytical capabilities keep pace. However, expedient method development that fails to account for the diastereomeric complexity of this alkaloid class risks introducing reporting inaccuracies, a challenge compounded by the scarcity of commercially available reference standards for many mitragynoid analytes. While minor diastereomers are rarely targeted in standard toxicosurveillance, their potential to coelute and inflate reported concentrations of 7-hydroxymitragynine underscores the necessity of chromatographic separation. For the pseudoindoxyl scaffold specifically, if dynamic equilibrium renders diastereomers chromatographically indistinguishable under physiological conditions, consolidated reporting as "mitragynine pseudoindoxyl" may be scientifically justified.

References

1. Angyal, Péter, et al. "Total Synthesis and Structural Plasticity of Kratom Pseudoindoxyl Metabolites." Angewandte Chemie International Edition 62.35 (2023): e202303700.

Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

L-007

Prevalence of Kratom in Postmortem Cases, Impaired Drivers, and Drug-Facilitated Sexual Assault Cases within San Francisco

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Abstract

Introduction: Kratom is a psychoactive herbal extract that comes from the leaves of a tree native to Southeast Asia (*Mitragyna speciosa*). Kratom's effects are dose dependent. Anecdotally, users report that lower doses produce stimulant like effects and higher doses produce opioid effects. Kratom has become a topic of interest in recent years due to increased use in the United States and attempts to legislate the drug.

Objectives: This study characterizes the prevalence of Kratom, and concentration trends of its psychoactive components in forensic casework in San Francisco from 2017 January to 2025 December. This study also describes polysubstance patterns associated with its use.

Methods: The San Francisco Office of the Chief Medical Examiner (SFOCME) routinely performs comprehensive routine forensic toxicological analysis on all non-natural and sudden deaths, driving under the influence of drugs and/or alcohol (DUID), and drug-facilitated sexual assault (DFSA) cases. We retrospectively analyzed up to ninety-six (96) months of routine toxicological testing for all Mitragynine, 7-Hydroxymitragynine, Speciociliatine, Speciogynine, and Mitragynine Pseudoindoxyl positive postmortem (PM), DUID, and DFSA cases within the City and County of San Francisco, totaling 81 cases.

Results: Cases involving individuals who used kratom rose steadily from 2017 to 2025, especially when systematic testing for Mitragynine was included in all routine casework in 2021. Kratom use was only suspected in 6.2% of cases, so targeted analysis may have led to 94% of these kratom-positive cases unreported. The majority of cases (n=44) were accidental drug overdose deaths (AOD). Mitragynine was present in all 81 cases with concentrations ranging from <5 ng/mL to >1,250 ng/mL in PM cases and < 5 ng/mL to 590 ng/mL in DUI and DFSA cases, respectively. 7-Hydroxymitragynine, Speciociliatine, Speciogynine, and Mitragynine Pseudoindoxyl were added to routing casework in 2025 and have been found in 24-38% of cases since implementation.

Of these 81 cases, the most common user of kratom was a biological male of White non-Hispanic descent (78% of cases). The most common age range for a person using kratom was 30-39 years (33% of cases). The most prominent co-occurring psychoactive substances were Opioids and Stimulants which were detected in 57% of cases, respectively. Overall, 66% of cases had 3 or more drug classes present.

Discussion: Kratom products are becoming more prevalent as a drug of abuse in San Francisco and frequently co-occur with other recreational and prescription drugs. The increase in Kratom-positive cases since the addition of Mitragynine and other kratom components to routine casework can indicate that Kratom positive cases may go undetected if the drug is not part of routine testing. Rising prevalence, a wide concentration range among users,

demographic information, and identification of psychoactive components in polysubstance cases can have implications for overdose risk, forensic surveillance, and public health policy.

Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

L-008

Early Identification of N-Propionitrile Chlorphine, aka cychlorphine, in a Western U.S. Jurisdiction

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Abstract

Introduction: The continued evolution and spread of novel synthetic opioids (NSO) continue to challenge forensic detection, interpretation, and public health response. N-propionitrile chlorphine, aka cychlorphine, a recently observed NSO, is suggested to be ten times more potent than fentanyl. It has limited published data, particularly within western U.S. jurisdictions. In April 2026, the Northern California DEA recently produced an alert indicating detection in seized material. In San Francisco, it has been monitored in forensic casework since 2025, with first detections in the same month of April, 2026.

Objectives: This study aimed to describe the initial detection of N-propionitrile chlorphine in forensic casework from San Francisco during 2026, and to characterize the range of case types and general toxicological features associated with its presence.

Methods: A retrospective review was performed on forensic toxicology casework received since N-propionitrile chlorphine's inclusion in routine testing (since January 1, 2025. Toxicological analysis utilized a comprehensive and validated workflow incorporating qualitative confirmation by liquid chromatography-quadrupole time-of-flight mass spectrometry (LC-QTOF-MS), with targeted quantitation by standard addition where applicable. Collected data included demographic characteristics, manner and cause of death when relevant, specimen type, analyte concentrations when available, and co-detected substances.

Results: N-propionitrile chlorphine was identified in four cases, all in the same month of its first detection, that of April 2026. The cases spanned multiple forensic contexts, including one sexual assault, one suspected overdose fatality, and two driving under the influence investigations. Detections occurred in polysubstance settings, namely with both novel and medicinal benzodiazepines and another nitazene NSO.

Discussion: The identification of N-propionitrile chlorphine across diverse case types within a short timeframe suggests rapid introduction into the local drug supply. Further, its presence across fatal accidental overdose, impaired driving, and sexual assault casework highlights its broader use in the community. These findings represent an early characterization of this NSO in a U.S. Western jurisdiction and underscore the importance of comprehensive toxicological screening and ongoing surveillance. As testing for this compound continues in routine casework, additional detections are anticipated based on emerging trends.

Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

L-009

Identification and Quantification of Four Etomidate Analogues in Hair Samples by LC-MS/MS

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Abstract

Introduction: Following the scheduling of etomidate and its initial analogues (e.g., metomidate, propoxate, isopropoxate) in China, illicit manufacturers have rapidly shifted to synthesizing novel structural variants to circumvent legal restrictions, thereby posing substantial challenges for forensic toxicology laboratories. Recently, four emerging etomidate analogues— ABP-700 (cyclopropyl-methoxycarbonyl metomidate), 2,6-dichloro-3-fluoro-etomidate, 4F-metomidate (4-fluorometomidate), and 4F-etomidate (4-fluoroetomidate), have recently been identified in seized e-cigarette liquids and biological specimens. Although some of these compounds were originally developed as "soft" anesthetic candidates, their pharmacological and toxicological profiles in the context of recreational abuse remain largely uncharacterized.

Objectives: This study aimed to identify four emerging etomidate analogues in seized e-cigarette liquids and to develop and validate a sensitive ultra-performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS) method for their simultaneous quantification in human hair samples.

Methods: Four seized e-cigarette liquid samples, each containing a different etomidate analogue, were analyzed by gas chromatography-mass spectrometry (GC-MS) and liquid chromatography-high resolution mass spectrometry (LC-HRMS), with elemental composition and structural confirmation achieved by comparison against certified reference standards.

Hair samples were collected from 11 suspected abusers who used e-cigarette liquids containing etomidate analogues and subsequently analyzed using the validated UPLC-MS/MS method. The hair samples were washed, segmented, and pulverized via cryogenic grinding. A precisely weighed 20 mg aliquot of hair powder was extracted using methanol ultrasonication with etomidate-d_5 as the internal standard (IS). Chromatographic separation was performed on an Acquity UPLC BEH C18 column (100 mm × 2.1 mm, 1.7 μm particle size) using gradient elution with acetonitrile and ammonium acetate buffer. Detection was carried out using a Waters Xevo TQ-S micro mass spectrometer operated in positive electrospray ionization (ESI+) and multiple reaction monitoring (MRM) mode. The method was comprehensively validated for selectivity, linearity, precision, accuracy, extraction recovery, and matrix effects.

Results: GC-MS and LC-HRMS analyses of four seized e-cigarette liquid samples confirmed the presence of ABP-700, 2,6-dichloro-3-fluoro-etomidate, 4F-metomidate, and 4F-etomidate, respectively. The validated UPLC-MS/MS method exhibited excellent linearity (r^2 >0.99), with limits of detection (LOD) and lower limits of quantification (LLOQ) of 0.005 ng/mg and 0.01 ng/mg, respectively. The method exhibited satisfactory analytical performance for all analytes, with intra- and inter-day precision below 10%, accuracy within ±10%, extraction recoveries of 96.2–112%, and matrix effects of 81.6–116%.

Application of the method to 11 hair samples collected from suspected abusers revealed concentration ranges of 0.02–0.11 ng/mg for ABP-700 (n = 2), 0.13 ng/mg for 2,6-dichloro-3-fluoro-etomidate (n = 1), 3.38–241 ng/mg for 4F-metomidate (n = 9), and 0.037–35.1 ng/mg for 4F-etomidate (n = 9).

Discussion: This study presents the first validated LC-MS/MS method for the quantification of four emerging etomidate analogues in hair matrix. Authentic case data confirm that these novel substances have already infiltrated the illicit drug market. As etomidate analogues continue to evolve, the analytical approach described here provides a valuable tool for the detection, monitoring, and forensic investigation of emerging psychoactive substances.

Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

L-010

Designer Benzodiazepines – Is There a Need to Validate a Quantitative Method

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Abstract

Introduction: Clandestine chemists can easily create new analogues by making slight alterations to a known synthetic drug molecule faster than most forensic laboratories can validate a new method for synthetic drugs like designer benzodiazepines (DBZDs). The problem is that many labs do not have the time and/or resources available to validate a new quantitative method for each new synthetic drug that emerges in the time frame that the drug is being consumed. In most DBZD-related cases, there is a concurrent presence of other drug classes such as opioids, THC, and licit benzodiazepines (BZDs). With the involvement of other drug classes, knowing the exact quantitation of a DBZDs in a case might not be important.

Objectives: To investigate the need to validate a quantitation method for DBZDs with a comprehensive casework review.

Methods: 604 cases involving DBZDs were reviewed to identify the presence of additional drug classes. Prevalence statistics were collected for 29 DBZDs: bromazepam, clobazam, clonazolam, cloniprazepam, diclazepam, etizolam, estazolam, flualprazolam, flubromazepam, flubromazolam, flurazepam, meclonazepam, n-desmethylflunitrazepam, nifoxipam, nimetazepam, prazepam, pyrazolam, tetrazepam, adinazolam, bentazepam, flunitrazolam, ketazolam, nitrazolam, bromazolam, cinazepam, phenazolam, difludiazepam, tofisopam, and fluclotizolam. All cases were screened by enzyme-linked immunosorbent assay for drugs of abuse classes which included amphetamine, methamphetamine, cannabinoids, opiates, benzodiazepines, cocaine, oxycodone, and fentanyl. All cases were also analysed by liquid chromatography quadrupole time of flight mass spectrometry for confirmation for over 400 compounds including illicit, prescription, over the counter, and novel psychoactive substances (including the 29 DBZDs).

Results: From 2020 to present day, 29,931 cases have been analysed by the Tarrant County Medical Examiner’s Office (TCME) with 604 (2.0%) confirmed as DBZDs positive. Approximately eighty percent of DBZD cases were coupled with opioids (33.2%), cannabinoids (59.4%) and/or licit BZDs (21.8%). In 4.1 % of cases, DBZDs were the sole positive finding with 2020 having 13 DBZDs only cases and the rest of the years having 0-3 cases of DBZDs only cases. As of April 2026, there have been 13 cases of DBZDs; with 1 being DBZDs only.

Discussion: Toxicology revealed that 94.6 % of DBZDs driving under the influence of drugs (DUID) cases had other impairing drugs present; similarly, 98.0 % of DBZDs post-mortem (PM) cases involved other drugs that contributed to the cause of death (COD). Total DBZD confirmed cases decreased from 4.7% (2020) to 1.3% (2025). This fleeting nature of DBZDs combine with the involvement of other drugs indicates that developing and validating a quantitative method for DBZDs offers low utility; therefore, qualitative confirmation should be sufficient. In DBZDs cases, quantitation is less critical for testimony than the functional effects of the drug. Because DBZDs act similarly to licit benzodiazepines, an analyst should focus on testimony regarding the possible physiological effects present at the time of the stop rather than a specific numerical amount. In PM cases, pathologists frequently certify the COD as mixed drug intoxication, regardless of the

DBZD concentration. Thus, depending on a laboratories workflow and customer request, DBZD quantitative validation may not be necessary.

Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

L-011

Identification of ADB-5en-HEXINACA and Other Synthetic Cannabinoids by GC-MS in Adulterated Low-THC Cannabis Products

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Abstract

Introduction: The presence of the emerging synthetic cannabinoid receptor agonist (SCRA) ADB-5en-HEXINACA in adulterated low-THC cannabis products has recently been reported on the European market. ADB-5en-HEXINACA (also known as ADMB-5en-HEXINACA or ADMB-5en-HINACA) is a synthetic cannabinoid belonging to the 3-carboxyindazole subgroup, with structural similarity to ADB-HEXINACA and ADB-4en-PINACA, for which official notifications were issued in 2021. To the best of our knowledge, no mass spectrometry data are currently available in the recent literature regarding the detection of this molecule in seized samples.

Objectives: In order to monitor the spread of cannabis products in our territory, a total of 304 cannabis-derived samples (118 plant materials and 186 resins) seized between January and April 2026 in the western Piedmont area (Italy) were analyzed.

Methods: General screening analyses for traditional and synthetic cannabinoids were performed using a GC–MS system operating in full scan mode (40–600 amu). THC, CBD, and CBN were quantified by means of a specific GC–SIM–MS protocol routinely employed in our laboratory. Qualitative identification of the compounds was carried out using reference standards or by comparing the acquired full scan spectra with those recorded in updated spectral libraries (SWGDRUG v. 3.14 and Cayman Spectra Library v. 10122025), applying a matching criterion of ≥80%.

Results: Overall, synthetic cannabinoids were detected in 20 out of 304 samples (6.6%). Considering only the 186 resin products, a positivity rate of 10.8% for SCRAs was observed. Several synthetic cannabinoids were identified, including MDMB-4en-PINACA (n=3), EDMB-4en-PINACA (n=4), MDMB-4en-PICA (n=1), MDMB-PINACA (n=3), ADB-PINACA (n=4), and ADB-5en-HEXINACA (n=7). As the latter molecule was not included in the updated spectral libraries, an electron ionization (EI) fragmentation study was performed to identify the most informative diagnostic ions. The presence of ADB-5en-HEXINACA in the seized material was confirmed by comparing the full scan spectra with those obtained from the analysis of a synthesized reference material. Regarding traditional cannabinoids, THC concentrations ranged from <0.2 to 1.6% w/w, while CBD was found at higher concentrations in all samples, specifically in the range of 2.9–31.1% w/w.

Discussion: The adulteration of low-THC cannabis products with synthetic cannabinoid receptor agonists is currently a widespread phenomenon. Since these substances are potentially more toxic than THC, their consumption poses a high risk of overdose for unaware users and may result in life-threatening conditions. This study confirms the presence on the market of CBD-prevalent cannabis products adulterated with multiple synthetic cannabinoids on the Italian territory, including the emerging compound ADB-5en-HEXINACA.

Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

L-012

Enzymatic Hydrolysis of Psilocin-*O*-glucuronide for Screening of Psilocin in Human Urine by Liquid Chromatography-Triple Quadrupole Tandem Mass Spectrometry

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Abstract

Introduction: Psilocin is a psychoactive indole alkaloid and metabolized to psilocin-*O*-glucuronide *in vivo* and excreted in urine. Therefore, efficient conversion of psilocin-*O*-glucuronide to free psilocin impacts urine psilocin testing accuracy. Despite the recent development of recombinant β-glucuronidases, little information on enzymatic hydrolysis of psilocin-*O*-glucuronide has been published since 2014. We hereby initiated a study and investigated hydrolysis of psilocin-*O*-glucuronide in human urine catalyzed by one conventional β-glucuronidase originating from *E. Coli* and three recombinant enzymes (*IMCSZyme*, Kura *BGTurbo*, and Kura *B-one*) as well as the effects of enzyme concentration, pH, incubation time, and incubation temperature on the hydrolysis efficiency.

Objectives: To provide quantifiable scientific references as a general guideline for other forensic toxicology laboratories to establish or optimize their own enzymatic psilocin-*O*-glucuronide hydrolysis methods and protocols.

Methods: Our LC-MS/MS-based screening method was validated in accordance with ANSI/ASB Standard 036. LOD is 0.2 ng/mL. Psilocin-d_{10} was utilized as the internal standard (IS). The concentrations of psilocin-*O*-glucuronide (equivalent to free psilocin) and the IS were 50 and 10 ng/mL, respectively. In brief, the buffer and the enzyme solutions were added to 50 µL of urine specimen containing psilocin-*O*-glucuronide and IS. Following incubation, the reaction mixture was centrifuged at 4,000 rpm for 5 min. The supernatant was then injected into the LC-MS/MS. The hydrolysis efficiency was calculated with the following formula: %hydrolysis=(A/B)×100%, where A is the averaged drug to IS response ratio in the hydrolyzed samples and B is the averaged theoretical drug to IS response ratio based on 100% conversion collected in the same run.

Results: The enzymatic hydrolysis rate trended higher with the increased enzyme concentration but was not proportional to enzyme concentration. The incubation temperature appeared to have more substantial impact than the incubation time. At 25, 40, and 50 °C, *E. Coli*, *BGTurbo*, and *B-one* offered more steady increase in the hydrolysis over 60 min while the performance of *IMCSZyme* slowed down after 30 min. *E. Col*i consistently offered slower conversion compared to that with the three recombinant enzymes. Additionally, except for *BGTurbo*, no other enzymes were able to achieve hydrolysis completion within 60 min. When temperature rose to 60 and 70 °C, hydrolysis catalyzed by the three recombinant enzymes was able to complete within 30 min, while *E. Coli* activity started declining at 70 °C. At 80 °C, all enzymes resulted in significantly diminished psilocin formation, while *IMCSZyme* exhibited the best resistance against temperature changes (Table 1). In addition, it was found that the low yield of psilocin generation at higher temperatures was due to reduced enzyme activity rather than thermo-decomposition of psilocin-*O*-glucuronide or the released psilocin.

Discussion: We present the first detailed study on recombinant β-glucuronidases induced enzymatic hydrolysis of psilocin-*O*-glucuronide in human urine in over 10 years. In summary, *BGTurbo* achieved the fastest hydrolysis completion at the lower temperatures (25-50 °C) while *IMCSZyme* offered the most consistent performance across a wide range of incubation temperatures (25-80 °C). These results provide quantifiable scientific references for forensic toxicology laboratories to establish or optimize their own hydrolysis methods.

Table 1. Effects of Incubation Temperature and Incubation Time on Psilocin-*O*-glucuronide Hydrolysis Efficiency (Conversion Rate)

Temperature (°C)			**25**			
Time (min)	*10*	*20*	*30*	*40*	*50*	*60*
E. Coli	7.69%	12.39%	20.55%	26.71%	29.89%	31.02%
IMCSzyme	58.77%	67.48%	74.75%	76.56%	75.39%	77.11%
BGTurbo	63.41%	69.32%	79.33%	85.71%	93.11%	96.21%
B-One	33.59%	57.46%	74.56%	79.63%	81.74%	84.15%
Temperature (°C)			**40**			
Time (min)	*10*	*20*	*30*	*40*	*50*	*60*
E. Coli	16.23%	26.96%	34.50%	37.33%	41.65%	44.02%
IMCSzyme	65.84%	76.07%	82.39%	85.39%	87.08%	89.16%
BGTurbo	77.05%	86.31%	96.20%	92.60%	99.98%	98.79%
B-One	61.42%	72.29%	81.85%	84.11%	86.97%	87.32%
Temperature (°C)			**50**			
Time (min)	*10*	*20*	*30*	*40*	*50*	*60*
E. Coli	33.21%	42.77%	51.72%	54.27%	57.69%	59.36%
IMCSzyme	78.69%	81.76%	85.40%	88.11%	90.27%	94.33%
BGTurbo	81.55%	84.90%	103.77%	109.61%	99.63%	106.24%
B-One	73.18%	76.25%	83.37%	85.74%	88.87%	92.43%
Temperature (°C)			**60**			
Time (min)	*10*	*20*	*30*	*40*	*50*	*60*
E. Coli	52.10%	67.09%	69.42%			
IMCSzyme	88.79%	94.71%	107.49%			
BGTurbo	97.51%	112.33%	102.59%			
B-One	87.54%	96.33%	104.42%			
Temperature (°C)			**70**			
Time (min)	*10*	*20*	*30*	*40*	*50*	*60*
E. Coli	47.36%	59.14%	50.37%			
IMCSzyme	105.10%	107.27%	102.89%			
BGTurbo	97.39%	93.77%	95.69%			
B-One	96.33%	106.52%	109.11%			
Temperature (°C)			**80**			
Time (min)	*10*	*20*	*30*	*40*	*50*	*60*
E. Coli	16.30%	19.22%	18.39%			
IMCSzyme	93.12%	90.67%	91.69%			
BGTurbo	44.69%	42.88%	46.41%			
B-One	82.52%	80.71%	80.53%			

Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

L-013

When the Sample Isn't Real: A Tiered Screening and LC-QTOF-MS/MS Confirmation Strategy for Detection of Diluted, Adulterated, Substituted, and Synthetic Urine Samples

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Abstract

Introduction: Specimen tampering remains a significant challenge for workplace drug testing, forensic toxicology, clinical laboratories, and anti-doping programs. Strategies including dilution, chemical adulteration, specimen substitution, and the use of synthetic urine products continue to evolve, reducing the effectiveness of conventional specimen validity testing and increasing the risk of undetected manipulation. Commercially available synthetic urine products are increasingly sophisticated and are designed to closely mimic the physicochemical properties of authentic urine, necessitating more robust approaches to specimen authenticity assessment.

Objectives: To evaluate common forms of specimen tampering, including dilution, adulteration, substitution, and synthetic urine use; assess the limitations of routine specimen validity testing; and develop a practical, cost-effective screening and confirmatory workflow to improve specimen authenticity assessment.

Methods: A systematic evaluation of commercially available synthetic urine products, authentic human urine specimens, and real-world casework samples was conducted. A tiered screening protocol incorporating routine specimen validity parameters, including creatinine, oxidant, pH, specific gravity, and amylase, was evaluated. The performance of amylase as an endogenous marker of specimen authenticity was compared with that of gamma-Glutamyl Transferase (GGT). Samples meeting predefined criteria for suspected tampering were escalated for confirmation using a novel LC-QTOF-MS/MS method developed and validated to differentiate authentic urine from substituted and synthetic specimens based on distinct endogenous chemical signatures, including 24 indigenous urinary compounds not replicated in commercial synthetic urine products. More than 100 authentic, synthetic, and casework specimens were evaluated to assess method performance.

Results: Routine specimen validity testing effectively identified many diluted and chemically adulterated samples but demonstrated reduced performance for adulterated, substituted and synthetic urine specimens designed to mimic authentic urinary chemistry. Commercial synthetic urine products were found to contain increasingly complex formulations incorporating urea, creatinine, uric acid, salts, minerals, pH modifiers, dyes, and temperature-maintaining devices intended to evade routine validity testing. The enhanced screening protocol improved the identification of suspicious specimens requiring further investigation. The LC-QTOF-MS/MS confirmatory method reliably differentiated authentic urine from adulterated, substituted and synthetic specimens, including products designed to evade conventional screening approaches. Application to casework identified previously unrecognised specimen substitutions and confirmed suspected tampering events.

Discussion: These findings demonstrate that conventional specimen validity testing alone is insufficient to address increasingly sophisticated tampering strategies. A tiered approach combining enhanced chemistry screening with targeted LC-QTOF-MS/MS confirmation for qualifying samples provides a practical and operationally feasible framework for comprehensive specimen validity assessment. The proposed workflow improves the detection of dilution, adulteration, substitution, and synthetic urine use while strengthening confidence in specimen authenticity determinations. This approach addresses important gaps in current testing practices and offers a defensible strategy for implementation across forensic, workplace, clinical, and anti-doping testing environments.

Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

L-014

Sensitive Detection of the Alcohol Biomarker Ethylated Phosphorylcholine (EtOChoP) in Urine by a Reversed-Gradient Reversed-Phase LC-MS/MS and Comparison to Elimination of EtG in Urine in a Pilot Single-Subject Drinking Study

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Abstract

Introduction: Ethylated phosphorylcholine (EtOChoP) has recently emerged as a potential novel biomarker of alcohol consumption. Preliminary investigations demonstrated detection of EtOChoP in blood matrices following ethanol intake; however, urinary detection was limited by substantial matrix effects. This study describes the development of a sensitive LC–MS/MS method for urinary EtOChoP detection and characterizes its elimination profile following controlled ethanol administration in comparison with ethyl glucuronide (EtG).

Objectives: To develop a LC–MS/MS method for EtOChoP in urine and to characterize its urinary detection and elimination profile after controlled low-dose ethanol administration in comparison to EtG.

Methods: A controlled single-subject drinking study was conducted in a volunteer (male, 80 kg) with social drinking habits. The participant ingested 30 g of ethanol within a 2-hour period, corresponding to an estimated blood alcohol concentration of 0.42–0.52‰. A total of 13 urine samples were periodically collected over 104 h following the last ethanol intake.

Urine samples were prepared by protein precipitation with ice-cold acetonitrile.

EtG was analyzed using an established LC–MS/MS method by *Neumann et al*. with minor optimizations. For EtOChoP, a novel LC–MS/MS method was developed. Chromatographic separation was achieved using a Kinetex® 2.6 µm F5 column under atypical reversed-phase conditions with a reversed gradient. The initial mobile phase consisted of 5% solvent A (water + 0.1% formic acid) and 95% solvent B (acetonitrile + 0.1% formic acid). A linear gradient was applied over 3 minutes to reach 95% solvent A and 5% solvent B. Eight calibration standards (1.25–250 ng/mL) were prepared by spiking blank urine with EtOChoP. Quantification of EtOChoP was performed using a deuterated internal standard (EtOChoP-D_5). Both compounds were synthesized by Dr. Vladimir Belov (MPI, Göttingen, Germany).

Results: EtOChoP was first detectable in urine 0.5 h after the last alcohol intake at a concentration of 3.65 ng/mL which corresponds to the maximum observed concentration. Subsequently, a continuous decline was observed: 2.91 ng/ml (4 h), 1.75 ng/mL (8 h), with the last detectable EtOChoP level measured at 11 h (0.99 ng/mL). In comparison, EtG remained detectable over the entire observation period with a concentration of 6410 ng/mL (0.5 h), 18900 ng/mL (4 h), 13300 ng/mL (8 h), 5720 ng/mL (11 h), 699 ng/mL (19 h), 248 ng/mL (24 h), 162 ng/mL (32 h), 22.4 ng/mL (44 h), 15.2 ng/mL (56 h), 14.7 ng/mL (68 h), 4.39 ng/mL (80 h), 1.43 ng/mL (92 h), and 1.0 ng/mL (104 h).

Discussion: This study demonstrates successful urinary detection of EtOChoP using a novel reversed-gradient reversed-phase LC–MS/MS method that effectively mitigated matrix interference. Compared with EtG, EtOChoP exhibited a markedly shorter detection window and rapid elimination profile following a single low-dose ethanol exposure. These preliminary findings suggest that EtOChoP may serve as a biomarker of very recent alcohol consumption rather than prolonged abstinence monitoring. Additional studies are needed to characterize interindividual variability, biological formation pathways, and elimination kinetics under conditions of chronic or excessive alcohol use.

Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

L-015

Drug Screen Suite: A Streamlined LC-HRMS Workflow for Routine and Post-Mortem Toxicological Analysis

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Abstract

Introduction: In high-throughput forensic laboratories, robust, easy-to-use solutions are essential, as data review during screening is a bottleneck delay casework. Liquid chromatography high-resolution mass spectrometry (LC-HRMS) remains a approach for comprehensive toxicological screening, enabling confident identification based on retention time, accurate mass, and MS/MS spectra. Continuous emergence of new psychoactive substances (NPS) demands workflows that can be updated rapidly.

The Drug Screen Suite (DSS) addresses these challenges by offering a fully integrated, end-to-end solution for routine toxicological screening, from sample preparation to reporting. This study demonstrates robust, sensitive, and specific performance in complex, matrix-rich samples, confirming its suitability for routine forensic workflows.

Objectives: Evaluation of a streamlined LC-HR-MS approach for screening samples in forensic casework.

Methods: Blood samples were prepared by protein precipitation (required due to chlorobutane incompatibility of some analytes) and, when needed, by alkaline liquid-liquid extraction. Vitreous humor was processed using a two-step SPE (Biotage Isolute HCX, 100 mg/3 mL), while urine samples were extracted with 500 µL cold acetonitrile. Extracts were evaporated under nitrogen and reconstituted in 25 µL of mobile phase A:B (50:50).

Samples were analyzed using the DSS, consisting of an Elute+ UHPLC coupled to a compact QTOF mass spectrometer (Bruker) operated in positive mode with auto MS/MS. Data evaluation used both an internal toxicological library and the Maurer/Meyer/Helfer/Weber LC-HRMS/MS library. Compound identification was based on library matching, retention time, and mass accuracy, applying an Effective Purity Score > 600. Automated processing included mass recalibration, feature extraction, spectral matching, and reporting.

Results: Proficiency tests issued by the Society of Toxicological and Forensic Chemistry (GTFCh) were evaluated, including urine tests for screening, abstinence monitoring, and unknown analysis (serum/urine), as well as serum proficiency tests for benzodiazepines, neuroleptics, and narcotics. All compounds contained in the library were identified using the DSS with auto MS/MS, except for norbuprenorphine at 15 µg/L.

To assess performance under high matrix complexity, post-mortem casework samples (5 samples in different matrices) were reanalyzed. These cases had previously been screened using routine toxicological methods, including immunoassays and Toxtyper. The case involved a driver fatally injured in a traffic accident. The DSS detected 21 substances and/or metabolites across multiple matrices, including femoral blood, vitreous humor, and urine. Results were consistent with routine

qualitative findings. Demonstrating its wide dynamic range, the DSS enabled quantitation of nine compounds in femoral blood from 1.0 to 350 µg/L.

In contrast, analysis of a post-mortem case with no toxicological findings yielded no detections. Despite the high matrix load typical of post-mortem samples, no false positives were observed in any matrix, demonstrating high analytical selectivity.

Discussion: The data demonstrate that DSS provides reliable and selective identification of a broad range of substances in both proficiency tests and complex post-mortem matrices. The strong agreement with routine screening and confirmatory results, combined with the absence of false positives even under high matrix load, highlights method robustness and forensic suitability. DSS improves workflows through higher sensitivity and untargeted analysis, enabling retrospective data evaluation and deeper insights without remeasuring samples.

Disclosure

Yes, I, or a member of my immediate family, has a financial interest to disclose.

Conflict of Interest

Salary/Consultant

L-016

Recommendations for Expanding the Scope of an Existing LC-MS/MS Method

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Abstract

Introduction: Emerging drugs of abuse compounds are a consistent pain point for toxicology laboratories. There are often new analytes and novel psychoactive compounds (NPS) emerging every quarter. To stay current, it is important for labs to update their testing panel, but finding ways to keep methods up to date can be both time consuming and challenging. This work provides recommendations to make these updates easier. Using emerging compounds from the Center for Forensic Research and Education's (CFSRE) Scope Recommendations, 20 compounds were selected to be added to an existing LC-MS/MS method that contained 231 analytes. Recommendations range from analyte selection, metabolite considerations, as well as instrument method modifications and the use of a virtual chromatogram modeler.

Objectives: The objective of this work is to offer recommendations to testing laboratories on easier ways to expand their testing scope when constantly dealing with new and emerging compounds.

Methods: An existing LC-MS/MS screening method was chosen because of its extensive scope of 231 analytes. The method used a Biphenyl 100 x 2.1 mm, 2.7 um column paired with a Biphenyl Guard Column Cartridge 5 x 2.1 mm, 2.7 um. All existing analytes were separated using a 12-minute cycle time. Gradient conditions were used at 30 ˚C using water and methanol, both containing 0.1% formic acid and 2 mM ammonium formate. The 20 analytes were added to the method and tested under optimized method conditions first by using a virtual chromatogram modeler to test retention and separation of the new analytes. A few of the new analytes were isobars with existing analytes in the original method. For example, a-methyl acetyl Fentanyl from the expansion list and Fentanyl from the original list. Because of the different sets of isobars method parameters were also tested to ensure resolution. These method changes included modifications to the column temperature and gradient conditions. Any method changes would need re-validation, so the aim of this work is to present ways to add analytes with as little laboratory burden as possible. In addition to the mentioned recommendations, the use of a virtual chromatogram modeler will also be explored.

Results: Analyte selection was in accordance with the Scope Recommendations provided by the CFSRE. Twenty of the newest emerging compounds were tested on an existing screening method to provide an example of elution order as well as isobar separations. The use of a virtual chromatogram modeler can be beneficial in instances like these. Ease of use and application of the modeler was demonstrated. Due to specific isobar groups, additional modified method parameters were provided to address these separations as necessary. All additional analytes retained well on the existing method.

Discussion: Toxicology labs are constantly dealing with identification and testing of emerging compounds. It is often difficult and time-consuming to update existing methods. The recommendations included here in this work are intended to ease the burden of scope additions, while retaining the integrity of the existing method. Resources such as the CFSRE are extremely helpful tools when considering what additions should be added to the testing scope. This resource paired with the recommendations above should help with toxicology labs future scope expansions.

Disclosure

Yes, I, or a member of my immediate family, has a financial interest to disclose.

Conflict of Interest

Salary/Consultant

L-017

Evaluation of Blood Fentanyl Concentrations in Wisconsin Drug-Impaired Driving Cases from 2016 to 2025

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Abstract

Introduction: Beginning in 2016, the Wisconsin State Laboratory of Hygiene (WSLH) Forensic Toxicology Program, along with laboratories nationwide, observed a shift from traditional heroin to fentanyl in the illicit drug supply, resulting in increased prevalence of fentanyl in drug-impaired driving (DUID) casework. Fentanyl rapidly crosses the blood–brain barrier and exhibits high affinity for opioid receptors, making it 50 times more potent than heroin[1]. This abrupt change in the drug market exacerbated the opioid epidemic and led to a substantial increase in overdose and DUID events. Many blood fentanyl concentrations observed during this period are theoretically fatal in opioid-naïve individuals.

In response to the continued increase in fentanyl-positive cases, WSLH revised its drug cancellation policy on February 1, 2025, discontinuing the cancellation of fentanyl quantitation for any reason. This change was implemented to provide meaningful concentration data to stakeholders and to support the public health mission of WSLH.

Objectives: The objective of this study was to evaluate trends in reported blood fentanyl concentrations in WSLH operating while intoxicated (OWI) casework from 2016 through 2025.

Methods: All OWI cases with a reported whole blood fentanyl concentration from 2016 to 2025 were included. Annual average, median, minimum, and maximum fentanyl concentrations were calculated and compared across years.

Results: A total of 3,607 OWI cases with a reported whole blood fentanyl concentration were examined. The average blood fentanyl concentration increased by approximately 50% from 2016 to 2025. The highest annual average concentration occurred in 2023 (14 ng/mL), while the highest individual concentration (480 ng/mL) was reported in 2022. Median fentanyl concentrations increased gradually over the study period, from 5.6 ng/mL in 2016 to 7.3 ng/mL in 2025.

Discussion: Fentanyl-positive DUID cases reported by WSLH nearly quadrupled between 2015 and 2016 and continued to rise, reaching a peak 20-fold increase in 2020. Throughout the past decade, fentanyl has remained the most commonly identified opioid among recreational users in OWI casework, with evidence of substantial tolerance development as reflected in increasing blood concentrations. Typical therapeutic fentanyl concentrations are <5.0 ng/mL[2]; however, by 2025, the average concentration observed in DUID cases was more than double this range (11.9 ng/mL). The progressive increase in blood fentanyl concentrations suggests a growing tolerance among users within Wisconsin's driving population.

Sources:

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Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

L-018

Assessment of Response of the Intoxilyzer® 9000 to Volatiles of Forensic Relevance

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Abstract

Introduction: The Intoxilyzer® 9000 is the newest approved instrument for evidential breath ethanol testing in the province of Ontario. There is currently no published literature addressing Intoxilyzer® 9000 interferents, so this study will serve to advance the findings from a previous evaluation of the instrument.

Objectives: To identify whether and at which concentrations volatiles of forensic relevance may produce false blood ethanol concentration readings and/or activate the "INTERFERENT DETECTED" message on the Intoxilyzer® 9000.

Methods: Three volatiles of forensic relevance were prepared as aqueous solutions at concentrations ranging from 5 mg/dL to 30 mg/dL for isopropanol and methanol and from 2.5 mg/dL to 30 mg/dL for acetone; aqueous ethanol concentrations ranging from 10 to 100 mg/dL were also prepared. Replicates of 30-45 measurements were made for each concentration using the Intoxilyzer® 9000, and the number of times an "INTERFERENT DETECTED" message occurred was recorded. Mixtures of either acetone, isopropanol or methanol were examined at various concentrations with ethanol concentrations of 50 and 80 mg/dL.

Results: No apparent blood ethanol concentrations were reported at acetone concentrations of 2.5 - 30 mg/dL. The maximum apparent blood ethanol concentration reported for isopropanol and methanol with activation of the "INTERFERENT DETECTED" message was 12 and 30 mg/dL, respectively; without activation of the message, the maximum corresponding concentrations were 8 and 11 mg/dL. The minimum concentration examined at which the "INTERFERENT DETECTED" message was activated in 100% of replicates was 5, 30, and 10 mg/dL for acetone, isopropanol, and methanol, respectively. The maximum concentration examined at which the "INTERFERENT DETECTED" message was not activated in any of the replicates was 10 and 5 mg/dL for isopropanol and methanol, respectively. Acetone concentrations of 5 mg/dL and higher produced the "INTERFERENT DETECTED" message on all replicates; 2.5 mg/dL acetone produced this message in some but not all replicates. Ethanol concentrations produced results as expected. Evaluation of mixtures of ethanol and the other volatiles is ongoing – those results will be included when these data are presented.

Discussion: Apparent blood ethanol concentrations increased with increasing isopropanol and methanol concentrations as did the frequency and number of the "INTERFERENT DETECTED" messages. Acetone did not produce apparent blood ethanol concentrations at each concentration studied and all concentrations produced an "INTERFERENT DETECTED" in at least some of the replicates. Overall, the potential for acetone, isopropanol or methanol to significantly contribute to the apparent blood ethanol concentration without activating the "INTERFERENT DETECTED" message is low. Any undetected contribution of isopropanol or methanol to the apparent blood ethanol concentration would be minimized by the current procedure in Ontario where all breath

test results are reported rounded down to the nearest multiple of 10 mg/dL and the lowest rounded down value is what is used for litigation. How the volatile concentrations examined in this study relate to forensic casework will be discussed.

Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

L-019

Evolving Trends in Impaired Driving: A Data-Driven Analysis

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Abstract

Introduction: Impaired driving remains a significant public safety concern in the U.S., contributing to crashes, injuries, and fatalities. While alcohol impairment is well characterized, drug-impaired driving is more complex due to the wide range of substances involved and the lack of a clear relationship between drug concentration and impairment. The impaired driving landscape continually evolves, with shifting drug use patterns and increasing poly-drug involvement. Standardized toxicological testing aligned with NSC-ADID and ASB recommendations provides a consistent framework to evaluate drug trends over time. Such data enable reliable assessment of drug prevalence, concentrations, and combinations, supporting identification of emerging threats, informing prevention and enforcement strategies, and guiding evidence-based policy to improve roadway safety.

Objectives: The objective of this project was to compile and analyze four years of toxicology data (2021-2024) from impaired driving cases, focusing on identifying trends in drug positivity rates, evaluating changes in drug concentrations, and assessing the prevalence of high frequency drug combinations.

Methods: Data were compiled from suspected DUID cases submitted to the laboratory between 2021 and 2024. For each analyte, mean, median, minimum, and maximum concentrations were calculated. Trends in percent positivity and average and median concentrations were evaluated annually and over time using simple linear regression. Model parameters were estimated using ordinary least squares (OLS), and statistical significance was assessed using a two-sided t-test under the null hypothesis that the slope equals zero. P-values were calculated using a normal approximation, and analyses were implemented in JavaScript.

Results: From 2021 to 2024, a total of 72,394 suspected DUID cases were evaluated, with annual case counts remaining relatively stable (16,994–19,081). Overall drug positivity was high year-to-year (89–95%). Ethanol was commonly detected and showed a modest but significant increase in positivity over time ($p = 0.047$, average four year positivity 63%), while concentrations remained stable (mean four-year average 158 mg/dL). Among depressants, alprazolam was the most frequently identified benzodiazepine (4.4% overall; $n = 3,238$), with stable positivity but increasing concentrations ($p < 0.001$), whereas clonazepam and lorazepam declined in positivity, with decreasing concentration trends.

Stimulant patterns diverged, with amphetamine and methamphetamine positivity decreasing (from 19 to 16% and 18 to 14%, respectively), while cocaine/benzoylecgonine increased (9% to 11%), alongside rising concentrations. Cannabinoids remained highly prevalent (average 45% positivity over four-year period for delta-9 THC), with slight declines over time but increasing concentrations (mean delta-9 THC up to 8.9 ng/mL). Opioid trends were mixed, with fentanyl, the most commonly detected opioid (10% overall), declining from 12% to 7.7%, while other opioids remained low (≤1%) and stable.

Discussion: These findings highlight an evolving impaired driving landscape marked by shifting substance use patterns. Drug positivity patterns in impaired driving cases continue to evolve, with shifting trends observed both within and across drug classes. While ethanol is frequently detected as a co-occurring substance, notable changes are seen among drugs, including divergent stimulant trends, high cannabinoid prevalence with increasing concentrations, and variable patterns among sedative-hypnotics and opioids. Overall, these results emphasize the need for ongoing toxicological surveillance to monitor trends and inform strategies, with 2025 data to be incorporated into the presentation.

Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

L-020

Enantioselective Determination of Amphetamine and Methamphetamine in Paired Postmortem Matrices by GC–MS/MS

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Abstract

Introduction: The enantioselective composition of amphetamine and methamphetamine is of interest, as the enantiomers show differences in their pharmacological effects. Numerous synthesis routes have been described, resulting either in racemic mixtures, non-racemic mixtures, or enantiopure products. In addition to their use as drugs of abuse, both substances also have therapeutic applications. Drugs containing amphetamine such as Attentin®, Adderall®, Elvanse® or Dexedrin® are commonly prescribed for the treatment of attention deficit hyperactivity disorder (ADHD). In Germany, however, no preparations based on methamphetamine are approved. Nevertheless, Eldepryl®, a selegiline-containing drug is available and is metabolized to R-methamphetamine and subsequently to R-amphetamine. In the United States methamphetamine itself is used therapeutically. Under the trade name Desoxyn®, it is prescribed for the treatment of ADHD and obesity. Several LC-MS/MS methods for chiral separation have already been reported, primarily using whole blood, oral fluid, urine or plasma/serum as analytical matrices. Despite this, gas chromatography remains standard practice in many laboratories.

Objectives: The aim of this study was to develop a method for enantioselective analysis of amphetamine and methamphetamine in various human matrices via GC-MS/MS.

Methods: Matrices of interest included body fluids such as femoral vein blood, cardiac blood, urine, cerebrospinal fluid, bile, as well as liver, brain and kidney, and stomach contents. The extraction of 100 µL or 100 mg was achieved using liquid-liquid extraction with ethyl acetate. Prior to this, stomach contents and tissue samples were homogenised using a bead mill. Derivatization with R-(–)-α-methoxy-α-trifluoromethylphenylacetylchlo ride (R-MTPACl) was carried out in a microwave, reducing processing time. The resulting diastereomers were separated by GC on a HP-5MS column and detected by electron impact ion source operating in multiple reaction monitoring.

Results: The method showed linearity from 5 to 500 ng/mL or ng/g per enantiomer in all matrices. Limits of detection ranged from 0.5–1.6 ng/mL or ng/g for R-/S-amphetamine and from 0.4–1.9 ng/mL or ng/g for R-/S-methamphetamine, while limits of quantification ranged from 1.2–5.0 ng/mL or ng/g for R-/S-amphetamine and from 3.0–4.8 ng/mL or ng/g for R-/S-methamphetamine. Intra- and inter-day precision (relative standard deviation) were below 12 %, and accuracy (bias) did not exceed 13 %. Extraction recoveries ranged from 88–100 % for amphetamine-enantiomers and from 15–104 % for methamphetamine-enantiomers. The method was applied to postmortem samples from 28 individuals collected during autopsies between 2022 and 2025. Cases in which only amphetamine (racemic) was detected included 3 individuals (11 %). In the remaining cases, methamphetamine and amphetamine were identified. Of these, 16 cases (57 %) contained only the S-enantiomers, whereas both R- and S-enantiomers were found in 9 cases (32 %).

Discussion: A simple and sensitive GC-MS/MS method for the determination of R- and S-amphetamine and R- and S-methamphetamine was successfully validated in multiple postmortem matrices. While analyte concentrations varied between matrices and cases, enantiomeric profiles remained consistent within each case, indicating that enantiomeric composition is largely preserved across postmortem matrices.

Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

L-021

Fatal Phencyclidine Intoxication

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Abstract

Introduction: Phencyclidine (PCP) is a dissociative anesthetic abused in localized areas of the United States, including Houston. Fatal PCP toxicity is rare; however, at sufficiently high doses, PCP can cause strokes, seizures, and arrhythmias. Smoked administration is most common, as leafy material, such as tobacco or cannabis, is saturated with a PCP solution, allowed to dry, then rolled into a cigarette.

A 47-year-old Black man was found unresponsive in bed. He had a history of prescription drug and alcohol abuse and remote gunshot wounds, resulting in difficulty ambulating. On the night before his death, the decedent appeared altered, with slurred speech and difficulty walking. On scene were bottles of ethanol, a muscle relaxant not prescribed to him, and a large plastic container described by the roommates as “detergent purchased from the side of the road.”

Objectives: Here we present a PCP fatality with suspected oral ingestion and unusually high concentration.

Methods: Comprehensive postmortem toxicology testing included volatiles by dual column headspace gas chromatography with flame ionization detection, an immunoassay drug screen for ten drugs/drug classes, a full scan gas chromatography/mass spectrometry (GC/MS) screen for alkaline-extractable drugs, a liquid chromatography quadrupole time-of-flight mass spectrometry screen, and vitreous chemistries.

Results: At autopsy, pulmonary edema and urinary retention were observed. The gastric mucosa exhibited normal rugal folds, but was markedly erythematous with associated areas of denudation. The stomach contents were notable for a strong chemical odor and two immiscible layers; the upper, amber-colored layer remained fluid at -20°C.

An unidentified volatile peak was detected on each column in femoral blood and vitreous humor. Relative retention times did not match difluoroethane, acetonitrile, ethyl acetate, toluene, acetaldehyde, or chloroethane. Relative retention time comparison to published works suggests possible diethyl ether, but confirmation was not performed.

PCP at greater than 2,000 ng/mL (~2,200 ng/mL) was the only drug detected in femoral blood. PCP was confirmed in stomach contents by the controlled substances laboratory.

The cause of death was classified as toxic effects of phencyclidine and the manner was accident.

Discussion: Previous literature frequently identifies PCP as an incidental finding in violent or non-drug related deaths; however, fatal PCP intoxications are possible, alone or in combination with other drugs. PCP concentrations greater than 2,000 ng/mL are thought to be fatal, as was observed in this case.

Our laboratory frequently encounters PCP in postmortem casework, but the case presented here is unusual. First, the gastric contents suggest an oral route of administration. Second, the unknown volatile peak in blood and vitreous humor has not been observed in other PCP cases.

These two anomalies are possibly related. Assuming this volatile is the delivery vehicle for PCP, much of the solvent would evaporate and/or pyrolyze in smoked PCP products. In contrast, similar losses would not be expected with direct, oral consumption of a PCP-solvent mixture. Unfortunately, scene evidence was not collected to confirm this theory. This case highlights the collaboration required from scene investigators, pathologists, and toxicologists for thorough medicolegal death investigation.

Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

L-022

The Detection of N-Propionitrile Orphine in a Post-Mortem Urine Sample in the United-Kingdom

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Abstract

Introduction: Novel synthetic opioids are an emerging and ever-changing field in post-mortem toxicology. Over the last few years, nitazene opioids have emerged in the drug supply and more recently, the presence of piperidine benzimidazolone ("Orphine") opioids. Like nitazenes, these compounds were developed in the 1960s and are also thought to be extremely potent. Here we present a case where a novel orphine, N-Propionitrile orphine was predicted and then detected in a urine sample as part of a coronial investigation.

Objectives: To predict and identify the presence of N-propionitrile orphine in post-mortem casework.

Methods: Fluoride oxalate preserved post-mortem femoral blood and urine were received for toxicology testing from an individual as part of a coronial investigation in December 2025. Blood and urine samples (500 µL) were combined with borate buffer (pH 10), metonitazene-d3 internal standard, and hexane:ethyl acetate (9:1, v/v) extraction solvent. Following mixing and centrifugation, the organic layer was collected, evaporated to dryness, and reconstituted in acetonitrile–water (1:1). Prepared extracts were transferred to autosampler vials for HR-MS analysis. Analysis was conducted using a Thermo Vanquish Flex UHPLC system coupled to an Exploris 120 Orbitrap high-resolution mass spectrometer (HR-MS) with a Thermo Accucore Phenyl Hexyl column with a gradient of 1% to 99% organic over 10 minutes, held for 1.5 mins then re-equilibrated for a total run time 15.5 minutes. Accurate mass measurements (±5 ppm), retention times, and MS/MS fragmentation spectra were evaluated. An N-propionitrile orphine standard was procured from Cayman Chemical.

Results: A compound consistent with N-propionitrile orphine was detected in the urine sample and confirmed through comparison with a reference standard. The compound was identified alongside N-propionitrile chlorphine and chlorphine. In the corresponding blood sample, N-propionitrile chlorphine and chlorphine were detected; however, N-propionitrile orphine was not observed.

Additional toxicological findings in urine indicated heroin use and the presence of medetomidine, N,N-dimethylaminoetonitazene, and ketamine, which were not detected in femoral blood. Cocaine, paracetamol, citalopram, mirtazapine, and olanzapine were detected in both matrices.

Discussion: Based on current understanding of halogenated orphine analogue metabolism, N-propionitrile orphine is unlikely to be a metabolite of N-propionitrile chlorphine. Its presence may more likely represent the use of impure precursor chemicals associated with the synthesis of N-propionitrile chlorphine or contamination (intentional or inadvertent) in a clandestine laboratory setting. Given the anticipated high potency of orphine analogues, the detection of this compound is of toxicological relevance. This case highlights the importance of proactive

identification strategies and continued surveillance for emerging synthetic opioids in post-mortem investigations.

Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

L-023

Sudden Death in the Context of Clenbuterol and Anabolic Steroid Use

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Abstract

Introduction: There is a growing trend among amateur athletes to use pharmacologically active substances to improve their physical appearance and performance. This practice, which may involve the use of high doses of various anabolic agents and medications, can be life-threatening and even fatal. Polypharmacy is common among steroid users for different purposes: to promote weight loss and muscle definition, or to prevent or counteract the known adverse effects of anabolic steroids. The detection in biological samples of medications –such as diuretics, hypoglycemic agents, antihypertensives, and selective estrogen receptor modulators used to treat female infertility or breast cancer– in the absence of medical indication in the case history may guide the toxicological investigation toward the analysis of anabolic substances, particularly when their use is not initially suspected.

Objectives: To present the post-mortem case of a 42-year-old man associated with the use of anabolic drugs. At the scene, hypodermic syringes, ten different anabolic steroids, and other drugs were found. At autopsy, external examination revealed a muscular body with minimal subcutaneous fat, and hypogonadism. Internal examination showed no significant findings. Histological analysis revealed concentric left ventricular hypertrophy, pneumonic foci, and liver congestion. Biological samples (femoral blood, urine and liver) were submitted for toxicological analysis.

Methods: In addition to routine toxicological analysis –ethanol and other volatile compounds, as well as screening of more than 700 analytes, including drugs of abuse, new psychoactive substances and medications (psychotropic and commonly used)–, a targeted analysis was performed to identify and quantify anabolic steroids, their esters and metabolites, as well as related substances such as clenbuterol, letrozole, clomiphene, and tamoxifen, using UPLC-QTOF (Sciex-Zeno-TOF-7600) and LC–MS/MS (Sciex-Exion-LC-QTrap-6500+).

Results: The following substances were detected in blood: clenbuterol (1.4 ng/mL), drostanolone (3.2 ng/mL), oxandrolone (1.2 ng/mL), stanozolol (9.3 ng/mL), and their metabolites, testosterone (T: 6.1 ng/mL), clomiphene (0.9 ng/mL), ephedrine (20 ng/mL) and metformin (330 ng/mL). The same compounds were identified in urine, where epitestosterone (E) was also detected, with a T/E ratio of 4.7 (Normal endogenous ratio <6). In liver, stanozolol and its metabolite, testosterone, clomiphene, ephedrine, metformin, and telmisartan were detected.

Discussion: Clenbuterol, a beta-selective adrenergic agonist with bronchodilator, lipolytic and anabolic effects, was detected in blood at a toxic level. Its use can cause significant adverse cardiovascular effects and electrolyte imbalance, among other serious complications. The use of three different anabolic steroids (drostanolone, oxandrolone, and stanozolol) was confirmed; the endogenous or exogenous origin of the detected testosterone could not be determined. Clenbuterol and the detected exogenous steroids are not authorized for therapeutic use in humans in many countries, including Spain. Clomiphene, telmisartan, and metformin had no

medical indication in this case. Considering all available evidence, the forensic pathologist concluded that the cause of death was multiorgan failure primarily due to the use of anabolic substances. We recommend testing biological samples for anabolic steroids when drugs commonly used by steroid abusers are detected and the case history does not support a medical indication for their use.

Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose

L-024

Elevated Postmortem THC Concentrations Following Traumatic Deaths

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Abstract

Introduction: With the publication of ASB's standard for scope and sensitivity in medicolegal death investigations (ANSI/ASB Standard 119, 2021), postmortem toxicology laboratories should include routine testing for cannabinoids. The standard requires cannabinoid testing in cases with a known anatomical cause of death as well as cases with a suspected toxicological cause of death. Laboratories adopting the standard that had previously not analyzed for THC may now be asked to interpret postmortem results. The Montgomery County Coroner's Office toxicology laboratory has tested THC for many years in postmortem cases and observed some exceedingly high concentrations in postmortem blood.

Objectives: The purpose of this research was to examine postmortem cases with elevated THC concentrations and based on the mechanism or cause of death, determine any correlations.

Methods: All postmortem cases from 2020 to 2023 with quantitative THC concentrations were selected. This included blood taken at autopsy, blood taken during tissue donation, and antemortem samples collected before death resulting in 1,366 postmortem cases. Case demographics and mechanisms of death characterized as either blunt force trauma (BFT), piercing trauma (PT), or other were recorded. BFT included cases that were motor-vehicle related or cases with crush-related trauma. Piercing trauma included gunshot wounds and stabbing deaths.

Results: Cases were differentiated by mechanisms of death with the following distribution: BFT – 248; PT – 271; other – 847. Table 1 shows descriptive statistics for the concentration of THC within the three groups. Student t-tests compared THC concentrations across groups and demonstrated that both trauma groups were significantly higher than the non-trauma group ($p < 0.05$). Comparison between the two trauma groups did not show any statistical significance ($p = 0.72$).

	BFT	PT	Other
Number (N)	248	271	847
[Average] (ng/mL)	16.6	15.6	9.4
[Median] (ng/mL)	7.9	7.0	4.0
[Minimum] (ng/mL)	1.0	1.0	1.0
[Maximum] (ng/mL)	200	513	166

Discussion: Following review of 1,366 postmortem cases, THC concentrations are statistically significantly higher in someone who has experienced trauma prior to their death. One possibility for higher concentrations of THC could be that fat depots are disrupted by the traumatic event, releasing stored THC into the blood. This post-traumatic release of THC then artificially elevates the concentration determined in postmortem specimens. Another possibility is postmortem redistribution of THC after death. Previous work has demonstrated that cardiac blood concentrations of THC increased after death. Finally, in trauma cases with large volume blood loss, any postmortem redistribution would be into a smaller amount of total blood which would artificially increase the THC concentration.

Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

L-025

Tissue Distribution of Daridorexant in a Forensic Autopsy Case

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Abstract

Introduction: Daridorexant is a dual orexin receptor antagonist (DORA) approved for the treatment of insomnia and is the most recent addition to this class of sedative-hypnotic agents. Use of DORAs has expanded in recent years, with global market growth from approximately USD 890 million in 2020 to USD 1.55 billion in 2025. Our department has previously reported forensic findings for earlier DORAs, including suvorexant and lemborexant. While analytical methods for daridorexant detection in blood and plasma have been described, no published data are currently available regarding postmortem daridorexant concentrations. Such data are essential for interpretation of toxicological findings in forensic autopsy cases.

Objectives: The objective of this study was to characterize postmortem daridorexant concentrations and tissue distribution in a forensic autopsy case and to provide reference data relevant to forensic toxicological interpretation.

Methods: Postmortem specimens from a forensic autopsy case in which the decedent had been prescribed QUVIVIQ® (daridorexant) were analyzed. Quantitative analysis was performed using liquid chromatography-tandem mass spectrometry (LC-MS/MS). The analytical procedure was adapted from a previously validated LC-MS/MS method developed for lemborexant, daridorexant's immediate DORA predecessor. Extraction was achieved using a modified micro-volume QuEChERS (Quick, Easy, Cheap, Efficient, Robust, and Safe) method. Chromatographic separation and detection were performed under optimized conditions using multiple reaction monitoring (MRM). Linearity, sensitivity, accuracy, precision, and matrix effects were evaluated in whole blood, with additional matrix effect assessment ongoing for other tissues.

Results: Daridorexant was detected and quantified in all collected autopsy specimens. Concentrations measured in right heart blood, left femoral blood, urine, and liver were 139, 196, 22, and 54 ng/ml or ng/g, respectively. Differences between central and peripheral blood concentrations were observed, with a central-to-peripheral blood ratio of 0.7. Liver concentrations were lower than corresponding blood concentrations, suggesting limited hepatic sequestration of parent daridorexant. No significant analytical interferences were identified. Acetaminophen, ketoprofen, and caffeine were also detected, none of which were present at elevated concentrations. Complete quantitative results, including concentrations for all collected postmortem specimens, will be presented.

Discussion: This case represents the first known report describing postmortem daridorexant concentrations and tissue distribution in a forensic autopsy setting. The findings provide initial insight into central-to-peripheral blood relationships and tissue affinity of daridorexant after death. Daridorexant is not believed to the be the sole cause of death in this case. These data contribute to the limited forensic literature on DORAs and will aid forensic toxicologists in the interpretation of daridorexant findings in future casework. Continued accumulation of postmortem daridorexant data will be necessary to establish interpretive reference ranges and better understand postmortem redistribution behavior.

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Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

L-026

The Final Dose: Postmortem Toxicology in Suicide Cases

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Abstract

Introduction: According to the American Foundation for Suicide Prevention (AFSP), suicide was the 11th leading cause of death in the United States in 2023. In Alabama, it was the 13th leading cause of death, ranking 23rd among states. Per the United States Centers for Disease Control and Prevention (CDC), the crude death rate for suicides in Alabama in 2024 was between 16.2-16.7 deaths per 100,000 people, with the age-adjusted death rate between 15.9-16.5. While drug use is a known risk factor, it can be either the method of suicide or a contextual factor.

Objectives: To investigate the prevalence of drugs in suicide cases analyzed by the Alabama Department of Forensic Sciences and reported in 2025.

Methods: Suicide cases were evaluated for the year 2025. Cases were screened by enzyme immunoassay using either a Tecan Freedom Evo75 with Immunalysis reagents or a Randox Evidence+ Analyzer with Drugs of Abuse Whole Blood Array biochip technology. Confirmation was conducted using the following instruments: Agilent 8890 Gas Chromatograph, 5977B Mass Spectrometer; Agilent Liquid Chromatograph (1260 or 1290), Agilent Tandem Mass Spectrometer (6460, 6470, or Ultivo). As of February 2025, cases that were negative after targeted confirmations underwent expanded testing, including screening using an Agilent 1290 Liquid Chromatograph, 6545 Quadrupole Time of Flight. Considerations for investigation include the subject demographics and the drugs detected in overdose (OD) versus non-OD suicides.

Results: In 2025, 3,079 death cases underwent toxicological analysis and were reported. Of those cases, 10% (n=304) were ruled a suicide: 13% ODs and 87% non-OD. Both groups were predominately male and disproportionately white individuals. The age range for ODs was 15-79 years, with the majority ranging from 40-59. The age range for non-ODs was 13-94 years, with the majority ranging from 20-39. The top five drug classes detected in ODs were opioids (50%), ethanol (34%), antidepressants (32%), cannabinoids (24%), and benzodiazepines (21%), with 68% of the cases containing polydrug use. The top five drug classes detected in non-ODs were ethanol (34%), cannabinoids (32%), stimulants (19%), opioids (14%), and benzodiazepines (11%), with 38% of the cases containing polydrug use. Nineteen percent (19%) of non-OD suicides did not contain detectable drugs or ethanol.

Discussion: Drug findings represent one of many factors associated with suicide, alongside access to substances, means to methods, mental health, and life situations. It is unsurprising that the prevalence of opioids remains high in these case types, given the opioid epidemic. The prevalence of opioids and benzodiazepines in ODs were 3.6 and 1.9 times higher than non-ODs, respectively. The prevalence of cannabinoids was 1.3 times higher in non-ODs. Ethanol prevalence was similar between groups. Additionally, 81% of non-OD suicides contained one or more drugs; however, toxicological findings alone do not establish causation or intent. Actual OD suicide rates may be higher than reported, as they can be difficult to determine due to the complexity of drug

use and establishing an individual's intent. Future aims include tracking drug trends and suicide rates over multiple years to determine any correlations, especially as initiatives work to decrease both issues.

Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

L-027

Trends in Drug Positivity in Correctional Facility Deaths in Alabama

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Abstract

Introduction: The evolving landscape of drug use presents ongoing challenges in forensic toxicology, particularly with the emergence of novel psychoactive substances (NPS) and shifting patterns of polydrug use across Alabama. The Alabama Department of Forensic Sciences (ADFS) receives toxicological casework from across the state where these trends extend beyond the general population to incarcerated individuals from the Alabama Department of Corrections (ADOC).

Objectives: This study evaluates analyte positivity trends in decedents who, at the time of their death, were incarcerated across Alabama from 2020-2025, focusing on suspected overdose cases and deaths classified as unlawful, suspicious, or were otherwise not natural.

Methods: Comprehensive postmortem toxicological analysis was performed on samples obtained from incarcerated inmates by the ADFS toxicology section. Ethanol analysis was conducted using headspace gas chromatography with either mass spectrometric or flame ionization detection (HS/GC/MS or HS/GC/FID). Initial drug screening employed enzyme immunoassay platforms (Tecan Freedom Evo75 with Immunalysis reagents or Randox Evidence+ Analyzer with Drugs of Abuse Whole Blood Array biochip). Confirmation testing utilized gas chromatography-mass spectrometry (Agilent 8890 GC with 5977B MS) and liquid chromatography-mass spectrometry (Agilent 1260 or 1290 LC with 6460, 6470, or Ultivo MS) following liquid-liquid or solid-phase extraction. Beginning in February 2025, cases with negative drug results underwent expanded screening using liquid chromatography-quadrupole-time-of-flight mass spectrometry (Agilent 1290 LC with 6545 QTOF).

Results: A total of 710 cases were analyzed. Fentanyl was detected in 13% of cases, with 25% of those also positive for xylazine, consistent with emerging trends in opioid adulteration. Synthetic cannabinoids were identified in 10% of cases, with MDMB-4en-PINACA as the most prevalent. Other synthetic cannabinoids included 4F-MDMB-BUTINACA, 5F-ADB, and ADB-BUTINACA. Benzodiazepines were detected in 10% of cases, and stimulants (e.g., methamphetamine, and amphetamine) in 5%. Polydrug use was common, with frequent co-detection of opioids, benzodiazepines, and sedative-hypnotics. Regional variability in drug positivity was observed across facilities suggesting localized differences in drug supply or use patterns.

Discussion: Fentanyl remains a predominant contributor to drug positivity in correctional facility deaths, with increasing detection of xylazine and synthetic cannabinoids. The high prevalence of polydrug use underscores the complexity of these cases and the limitations of targeted testing alone. Expanded screening approaches, such as LC-QTOF, are critical for identifying emerging compounds and supporting comprehensive forensic and public health responses

Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

L-028

Thallium Intoxication vs. Differential Diagnosis of Guillain-Barré-Syndrome – Report of a Series of Poisonings

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Abstract

Introduction: Poisonings involving heavy metals are rare, but they continue to play a role in homicides and suicides. A proof of such poisonings is difficult because intoxications with these substances often result only in non-specific symptoms and the substances are not detectable in standard chemical-toxicological analysis. A series of thallium sulfate poisonings is presented here.

Objectives: To increase awareness of the relationship between neuropathological symptoms and possible heavy metals intoxications/exposure, especially thallium.

Methods: Based on the suspicion of thallium administration by one suspect in three cases, analyses of ante mortem serum and urine as well as post mortem blood and urine were performed using an Agilent 8900 ICP-MS/MS (Agilent Technologies, Waldbronn, Germany) after alkaline dilution with ammonia solution (Heitland et al. 2020). To hair and nail samples, 65% (V/V) nitric acid was added and the samples were digested for 30min at 90°C. An Agilent 7900 ICP-MS was used for analysis (Heitland et al. 2025). Tl was monitored at m/z=205; in both methods, 25 µg/L Rhodium was used as internal standard. Additionally, autopsy findings, medical records, and police investigation results were evaluated.

Results: Over a period of 1.5 years, three women were poisoned with thallium sulfate by the same man; two of those died. In the first case, the initial diagnosis was Guillain-Barré syndrome, however, the autopsy led to the postulation of thallium poisoning. Before the suspect could be further questioned about this, he also administered thallium sulfate to his pregnant partner. She survived the poisoning, but her child died a few months after birth. The second deceased was the grandmother of the pregnant partner, who was poisoned some months after his wife. Initially, this was rated as natural death and there was no suspicion of thallium poisoning. However, following the discovery of the other two cases of poisoning, an exhumation and a toxicological examination were performed.

Very high concentrations of thallium were detected in the postmortem femoral blood of the first deceased (8586 µg/L), as well as in the urine of (8330 µg/L) and in the nails (2.7 – 6.1 µg/g) of the second deceased individual, who had been exhumed. In the third victim, who survived, urine and serum samples as well as hair samples were tested for thallium over a period of several weeks. The initially very high concentrations (serum: 718 µg/L, urine: 8960 µg/L) could be reduced rapidly by administering an antidote and performing dialysis. In all cases, no toxicologically relevant substances were detected that could have explained the symptoms or contributed to the deaths.

Discussion: Both clinically treated women were initially diagnosed with Guillain-Barré syndrome, a fulminant acute autoimmune neuropathy. But later on, based on the typical symptoms of thallium poisoning —including hair loss after a prolonged latency period—

which differ from those of other heavy metals, the clinicians suspected thallium poisoning. In close cooperation between forensic institutes, state criminal investigation offices (Landeskriminalaemter), and prosecution as well as police, the accused could be convicted and was ultimately sentenced for two counts of murder, attempted murder, and attempted abortion. He did not comment on his motive during the trial.

Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

L-029

Forensic Evidence of a Significant Increase in Recreational Ketamine Use on the Spanish Mediterranean Coast

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Abstract

Introduction: Ketamine, a dissociative anesthetic with medical and veterinary applications, has increasingly emerged as a recreational drug in Europe over the last decade. Recent epidemiological indicators, including wastewater-based analyses, point to a sustained rise in ketamine consumption, particularly in urban and nightlife settings. In 2024, ketamine was one of the three most frequently detected NPS in drug samples worldwide, both as a knowingly consumed substance and as a component of polydrug mixtures marketed under labels such as “tusi” or “pink cocaine” (1,2,3). The present casework, handled by the Barcelona Department of the National Institute of Toxicology and Forensic Sciences (INTCF-Barcelona), provides an overview of ketamine detections in seized drugs and in biological samples submitted for forensic analysis. Additionally, this study serves as an indicator of market dynamics and consumption trends.

Objectives: The current work evaluates temporal trends in ketamine detection between 2021 and 2025 based on INTCF-Barcelona casuistry. The objectives were: (i) to quantify annual evolution of ketamine-positive cases in drug seizures and in biological forensic-related samples; (ii) to assess changes in the ketamine detection pattern; (iii) to characterize co-detection patterns with other psychoactive substances, particularly MDMA and 2C-B; and (iv) to assess the main component of drug mixtures known as “tusi” or “pink cocaine”.

Methods: A retrospective descriptive analysis was conducted on a total of 42,478 cases corresponding to all forensic requests processed by INTCF-Barcelona between 2021 and 2025. Cases were classified as drug seizures or biological forensic-related samples. Annual totals were recorded and ketamine-positive findings identified. In drug seizures, ketamine co-detection with MDMA and/or 2C-B was evaluated. In biological samples, special emphasis was placed on hair analyses, particularly those submitted with reported “tusi” consumption. Relative annual percentages were calculated to assess temporal trends.

Results: The total number of forensic requests increased from 6,679 in 2021 to 9,805 in 2025. Ketamine detections rose markedly in both drug seizures and biological samples over the studied period. In biological samples, ketamine-positive cases increased from approximately 6% in 2021 to 12% in 2025, with a sharp rise in hair samples associated with “tusi” use from 2023 onwards. In drug seizures, the total number of ketamine-positive cases increased more than fivefold (from 3.4% in 2021 to 13% in 2025). More than half of ketamine-positive seizures also contained MDMA, a proportion that increased progressively, while co-detection with 2C-B remained low (between 2% and 13% of cases).

Discussion: The increase in ketamine detections between 2021 and 2025 strongly suggests an expansion of ketamine availability and consumption on the Spanish Mediterranean coast. The frequent association of ketamine with MDMA in drug seizures, together with the high proportion of “tusi”-oriented hair samples containing ketamine (alone or in combination with other substances), indicates that ketamine, not 2C-B, is the core component of products marketed as “tusi” or “pink cocaine”.

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Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

L-030

Application of Fortified Hair as Quality Control Sample in Multi-Drug Analysis

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Abstract

Introduction: Quality control is essential in toxicology testing, as it ensures the validity and reliability of laboratory results. The quality control for hair analysis usually constitutes two parts: external and internal. External quality control involves participation in a proficiency testing program, in which the laboratory analyzes the hair specimens provided by an external proficiency testing provider. Spiking drug standards onto a drug-free hair matrix (hair blank) to mimic drug-containing hair is common practice for internal quality control. However, this approach does not fully replicate the distribution of drugs in authentic hair samples obtained from drug consumers and therefore cannot fully be used to replace the authentic hair samples.

Incorporation of authentic hair from drug consumers is ideal but supply is limited. Proficiency testing samples or commercially supplied hair controls offer a convenient alternative but they are not cost effective for laboratories that routinely analyze multiple hair batches daily. An alternative approach is to prepare in-house quality controls by soaking drug-free hair in a solution containing drug(s) of interest. This enables the incorporation of the drug(s) into the hair matrix and provides flexibility to customize the quality control samples according to the laboratory's specific requirements.

In this study, drug-free hair (A) was immersed in an aqueous solution containing 6-monoacetylmorphine (MAM), morphine, methamphetamine, methylenedioxymethamphetamine (MDMA), ketamine and flunitrazepam for five hours. These drugs represent the different categories of drugs of abuse analyzed by the laboratory.

Objectives: To evaluate and use the in-house prepared soaked hair quality control samples for routine analysis.

Methods: Following washing and drying, soaked hair (A1) was analysed by liquid chromatography-tandem mass spectrometry (LC-MS/MS) using six replicates to determine mean concentrations, standard deviation (SD) and coefficient of variation (CV). Additional data were collected over five months using LC-MS/MS (n = 39) and gas chromatography-tandem mass spectrometry (GC-MS/MS) (n = 17) to assess analytical variability and stability. To assess within-source and between-source variability, soaked hair (A2) was prepared from the same hair source as A1 at a different time, while two additional preparations (B1 and B2) were made from a separate hair source (B).

Results: A1 exhibited good stability over the study duration, with reproducible results across both methods. Concentration ranges were evaluated against three acceptance ranges: mean ± 2SD, mean ± 20 % and mean ± 30 %. The ± 30 % range was found to be the most appropriate, accommodating day-to-day analytical variation while maintaining acceptable precision. Differences in mean concentrations and acceptance ranges were observed between preparations and hair sources.

Discussion: The observed results were attributable to the inherent heterogeneity of hair matrices, and drug incorporation may vary depending on the specific characteristics of the hair source and the physicochemical properties of the individual drugs. These findings indicate that each soaked hair preparation requires the establishment of its own mean concentration and acceptance range for each individual drug. In-house soaked hair can serve as a suitable quality control sample for routine hair analysis provided that preparation-specific acceptance ranges have been established.

Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

L-031

Cross-Reactivity of Risperidone in Immunochemical Urine Screening for Fentanyl: A Case Study within the Italian Fentanyl Prevention Plan

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Abstract

Introduction: In response to the global opioid crisis, the Italian government implemented the Fentanyl Prevention Plan, involving the Ministry of Health and National Institute of Health to intensify the toxicological control of synthetic opioids in critical settings. Within this framework, an increasing number of false positive results from on-site screening tests for fentanyl have been reported, particularly from the psychiatric and emergency departments, suggesting the cross-reactivity of common psychiatric drugs with these immunological rapid tests.

Objectives: This study aimed to assess the potential cross-reactivity of risperidone and valproic acid with two commercially available immunochemical rapid screening devices for fentanyl in urine, commonly used in hospital settings. Furthermore, we evaluated the suitability of oral fluid device for fentanyl to a different matrix.

Methods: 9 urine samples that initially tested positive for fentanyl using AllTest device (Hangozhou AllTest Biotech) in the psychiatric department of a local health Unit were re-tested with JusChek+ device (ACRO Biotech). All samples underwent confirmatory GC-MS analysis for fentanyl and norfentanyl, after solid phase extraction (Bond Elut certify, Agilent) and derivatization with BSTFA+TMS at 70°C for 30minutes. In addition, a comprehensive LC-HRMS/MS qualitative analysis was conducted on all samples to identify the complete list of medications and related metabolites. A pool of ten pretested blank urine samples was spiked with risperidone, valproic acid and a mixture of both drugs at concentration of 1mg/ml and 0.5mg/ml. The AllTest device and the JusChek+ device for urine, as well as the JusCheck+ device for oral fluid, were tested according to the manufacturers' protocol. This research was funded by the Ministry of Health (eLEX-NPS project, CUP I85E25001230005).

Results: The nine clinical samples tested positive for fentanyl with AllTest device, whereas only 3 resulted positive also with JusChek+ urine device. Instrumental analysis by GC-MS confirmed that all the samples were negative for fentanyl and norfentanyl. Qualitative LC-HRMS/MS analyses revealed the concurrent presence of risperidone and valproic acid in all samples. In the spiked samples study, those containing only risperidone yielded positive results, while those spiked only with valproic acid remained negative across all the investigated devices. Samples spiked with the mixture of both drugs tested positive with all devices at all tested concentrations. Notably, the blank pooled urine sample yielded an inconclusive result when tested with the JusChek+ oral fluid device.

Discussion: The reporting system within the Fentanyl Prevention Plan played a fundamental role in identifying a critical diagnostic challenge in the hospital community, highlighting the need to further investigate previously undocumented cross-reactivity of prescription drugs with fentanyl-specific devices. In this study, we demonstrated that risperidone induces cross-reactivity in standard immunochemical rapid tests for fentanyl in urine. This interference is likely attributable to the para-substituted phenethyl moiety shared by the molecular structures of both fentanyl

and risperidone. This hypothesis is further supported by the fact that valproic acid, which has a fundamentally different molecular structure, exhibited no such cross-reactivity. In addition, the controversial results obtained with devices specific for oral fluid confirm the matrix-specificity of immunochemical rapid testing.

Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

L-032

Customized Toxicological Criteria for the Korean Population: Blood Concentrations and Clinical Symptoms in Acute Poisoning

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Abstract

Introduction: Current reference concentrations for determining drug intoxication are heavily based on foreign literature and individual case reports. These criteria often fail to reflect the ethnic, genetic, and environmental characteristics of the Korean population or adequately account for complex factors such as underlying diseases and poly-drug overdoses.

Objectives: This study aims to establish toxicological criteria tailored to Korean patients by analyzing blood drug concentrations in suspected acute drug intoxication cases presenting to emergency departments and correlating these levels with the manifestation of clinical symptoms.

Methods: In 2024, 480 positive blood samples from 742 suspected acute poisoning cases collected at regional centers were quantitatively analyzed, using liquid chromatography-tandem mass spectrometry following protein precipitation. Samples were obtained during the initial emergency department visit and accompanied by de-identified clinical information, including level of consciousness, vital signs, and laboratory findings. Cases were classified into therapeutic/non-toxic, poly-drug toxicity, and single toxicity groups by comparing blood drug concentrations with pharmacokinetic reference values from existing literature, while also considering clinical symptoms, co-ingested substances, and exposure to alcohol or carbon monoxide.

Results: The most frequently detected drugs were zolpidem, flunitrazepam (and its metabolites), and quetiapine. The therapeutic/non-toxic group accounted for 52.3% of cases, while the poly-drug toxicity group comprised 25.8%. In poly-drug toxicity cases, combinations such as antipsychotics with benzodiazepines or benzodiazepines with SSRIs (Selective Serotonin Reuptake Inhibitors) were common and frequently resulted in central nervous system depression. Among single toxicity cases, central nervous system depressants accounted for 75%. Notably, the blood concentration values observed in the single toxicity and poly-drug toxicity groups were similar. Most measured concentrations overlapped with values reported in the literature. However, toxic symptoms associated with sedatives and anticonvulsants appeared at higher blood concentrations than previously reported, whereas symptoms for antidepressants, antipsychotics, and antihypertensives occurred at lower concentrations than those documented in foreign studies. The study also proposes new reference concentration values for drugs with limited prior data, such as dapsone and doxofylline.

Discussion: The findings indicate that although foreign toxicological criteria are generally applicable, specific upward or downward adjustments may be necessary for the Korean population owing to differences in tolerance, metabolic enzyme activity, and individual sensitivity. The similarity in concentrations between single toxicity and poly-drug toxicity groups suggests that toxicity in poly-drug overdoses is primarily driven by overlapping pharmacological mechanisms rather than simple additive effects of drug concentrations. Despite limitations

including non-standardized symptom recording and relatively small sample sizes for certain drugs, this study provides an important foundation for developing a domestically tailored toxicological reference system. Future research should focus on increasing the number of cases and standardizing clinical symptom documentation to improve the reliability of concentration–symptom correlation analyses.

Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

P-100

Development and Validation of Automated Homogeneous Immunoassays for Opiates and Oxycodone in Oral Fluid: Integrating Structural Biology Practices into Antibody Engineering and Characterization

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Abstract

Introduction: Oral fluid testing has seen an increase in popularity in forensic toxicology and workplace drug screening due to its non-invasive collection and strong correlation with recent drug use. The rise in oral fluid samples demands higher throughput assays such as HIA (homogeneous immunoassay) that are compatible with automated chemistry analyzers. To meet the Substance Abuse and Mental Health Services Administration (SAMHSA) Oral Fluid Mandatory Guidelines (OFMG), assays must demonstrate low ng/mL sensitivity, validation with the intended collection devices, and cross-reactivity greater than 80% amongst grouped analytes such as oxycodone and oxymorphone or morphine and codeine.

Objectives: This study evaluates the performance of two newly developed HIAs for the detection of morphine, codeine, hydrocodone, hydromorphone, oxycodone, and oxymorphone. As a legacy anti-morphine antibody showed suboptimal cross-reactivity toward codeine and hydrocodone, we aimed to leverage an internally developed protein engineering pipeline to improve this profile. Furthermore, this study assesses the utility of a novel computational ligand-based predictor in characterizing antibody cross-reactivity during the assay development phase.

Methods: Two G6PDH-based immunoassays were developed for use on automated clinical chemistry analyzers. Both Opiates and Oxycodone/Oxymorphone HIAs were optimized for around 30 ng/mL cutoff. Samples were diluted 1:4 using Quantisal® oral fluid buffer (Immunalysis, Pomona, CA, USA) and Neogen® oral fluid buffer (Neogen, Lansing, MI, USA). A total of 100 de-identified human oral fluid samples were evaluated and confirmed by LC/MS. In addition, to streamline the characterization of these assays, a ligand-based cross-reactivity predictor—utilizing 2D and 3D molecular features—was employed to model potential interferences and cross-reactivity with structurally related opioids.

Results: The Oxycodone/Oxymorphone HIA achieved 92% sensitivity, 100% specificity, and an overall accuracy of 96% (AUC ROC = 0.982). The Opiates HIA achieved 90% sensitivity, 100% specificity, and an accuracy of 95% (AUC ROC = 1.0). Ninety compounds were screened for cross-reactivity, including common OTC medications, SAMHSA analytes, and various opioids. Protein engineering of the recombinant opiate antibody improved codeine and hydrocodone cross-reactivity to >80% relative to morphine. Computational prediction for cross-reactivity yielded a Pearson correlation coefficient of 0.704 on average with K-folded test data and identified 70 compounds with potentially significant cross-reactivity.

Discussion: These new HIAs are rapid (<8.5 minute) quantitative assays intended for high-volume oral fluid screening. The Opiates assay benefits from the consistency and fine-tuning of a re-engineered recombinant antibody. The integration of the ligand-based predictor allowed for an

in-silico exploration approach to cross-reactivity, reducing the need for exhaustive bench testing of rare interferences. These tools collectively enhance the speed and accuracy of toxicology assay development, ensuring compliance with evolving national standards.

Disclosure

Yes, I, or a member of my immediate family, has a financial interest to disclose.

Conflict of Interest

Stocks/Bonds, Salary

P-101

Development of an Automated Pretreatment Method for Cannabinoids and Related Compounds in Serum Using ATLAS-LEXT

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Abstract

Introduction: Certain cannabinoids are prohibited by law in Japan. In many other countries, by contrast, cannabis regulations have been relaxed, although concentration-based thresholds have been introduced to define legally permitted use. Therefore, blood analysis of cannabinoids is important in forensic toxicology. However, conventional pretreatment methods for blood samples, including liquid–liquid extraction (LLE) and solid-phase extraction (SPE), are labor-intensive, susceptible to human error, and may expose laboratory personnel to infection risk. Automated pretreatment may therefore improve safety, reduce operator dependency, and enhance analytical reproducibility.

Objectives: This study aimed to develop an automated SPE-based pretreatment method for the analysis of cannabinoids and related compounds in serum using ATLAS-LEXT.

Methods: Human serum samples were spiked with 15 cannabinoids to a final concentration of 5 ng/mL per analyte: Δ8-THC, Δ9-THC, 11-OH-Δ9-THC, Δ8-THC-COOH, Δ9-THC-COOH, Δ8-THCV, Δ9-THCV, Δ8-THCB, Δ9-THCB, Δ9-THCH, HHC, HHCH, HHCP, CBD, and CBN. Δ9-THC-COOH-d9 was used as the internal standard. Sample pretreatment was performed using the ATLAS-LEXT® automated sample preparation system (Shimadzu Trustech Corporation) with Oasis PRiME® HLB SPE cartridges (Waters Corporation). 20% aqueous methanol was used for washing and acetonitrile/methanol (9:1, v/v) for elution. Under extraction condition 1, the loading, washing, and elution steps were each centrifuged at 3000 rpm for 30 s. Under condition 2, all three steps were centrifuged at 1750 rpm for 180 s. Under condition 3, the loading step was centrifuged for 120 s, and the washing and elution steps were each centrifuged for 60 s, using stepwise centrifugation from 500 to 3000 rpm. Calibration curves were prepared from serum samples spiked at 1–25 ng/mL under condition 2, followed by LC–MS/MS analysis. Precision and trueness were evaluated at 2, 7.5, and 20 ng/mL (n = 4), and the limits of detection (LOD) and quantification (LOQ) were calculated using a regression-based approach.

Results: The extraction times for 10 samples were 104, 184, and 136 min under extraction conditions 1, 2, and 3, respectively. Condition 2 provided the highest recoveries for all 15 analytes, ranging from 12.1 to 92.7%. Matrix effects ranged from 56.9 to 131.9% (n = 4). Calibration curves showed good linearity for most analytes (R^2 = 0.98–1.00). Precision and trueness for most analytes were within ±15%. LODs ranged from 0.003 to 0.270 ng/mL, and LOQs ranged from 0.008 to 0.820 ng/mL.

Discussion: Although condition 2 required the longest processing time, it yielded the highest recoveries among the tested conditions and acceptable quantitative performance, and was therefore considered the optimal extraction condition. This method offers a safer and simpler workflow than manual procedures. However, analogs with longer alkyl side chains than THC showed lower recoveries, possibly due to stronger retention on the SPE sorbent as hydrophobicity increased. This may also explain the lower linearity observed in some analytes. SPE was selected

because its well-defined load–wash–elute workflow and the absence of a pH adjustment step made it suitable for automated handling; however, the results of this study suggest that it may not be the most suitable extraction method for certain analogs. Future studies should compare this method with LLE and evaluate it in whole blood.

Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

P-102

Evaluation of a Second-Generation Nitazene Lateral Flow Test Strip Using an Expanded Analogue Panel

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Abstract

Introduction: Nitazene opioids continue to emerge within illicit drug markets, presenting ongoing challenges for forensic toxicology due to their high potency, structural variability, and association with drug-related harm and fatalities. Rapid, presumptive screening tools for bulk drug samples such as lateral flow immunoassay (LFIA) test strips are increasingly used to support drug checking and forensic intelligence; however, independent evaluations have identified limitations in both sensitivity and selectivity.

Objectives: his study aimed to apply an established evaluation methodology to an improved second-generation nitazene testing strip (BTNX Rapid Response Nitazene Test Strips version 2.0), using an expanded panel of nitazene analogues. Performance was compared to the original BTNX Nitazene Test Strip with a particular focus on improvements relevant to cross-reactivity with desnitazenes and the reduction of false positives caused by caffeine.

Methods: An expanded panel of nitazene analogues (n= 69) and commonly encountered licit and illicit drugs and cutting agents (n= 96) were prepared at 100 µg/mL in aqueous solution. These test strips are intended for presumptive testing of bulk drug samples rather than biological specimens and no biological matrices were included in the evaluation. Test strips were used according to the manufacturer's instructions: briefly immersed (10-15 s), placed on a flat surface, and read within 10 min (typically after 1 min). Results were recorded as positive (one line) or negative (two lines).

Limits of detection (LOD) for nitazene analogues were determined by serial dilution in tap water. Tap water was used to mimic real-world conditions of end-users. Initial solutions (2000 ng/mL), based on reported isotonitazene sensitivity, were analysed in triplicate. Positive samples were diluted (1:2) until a negative result was obtained, while negative samples were tested at higher concentrations until positive. The LOD was defined as the lowest concentration at which all replicates were positive.

Results: The second-generation test strips demonstrated enhanced performance (LOD range = 500 to 5000 ng/mL) across the panel of 60 nitazene analogues with detection capability extended to include desnitazene analogues. This represents a key improvement over the original strips, which failed to detect this subclass. Notably, compounds such as metodesnitazene, which has been implicated in drug-related deaths in the United Kingdom, were successfully identified using the updated strips. Furthermore, the caffeine cross reactivity, observed with the original strips which resulted in false positive responses at high concentrations, was resolved in the updated products. Overall, the improved strips exhibited increased selectivity and a reduction in both false positive and false negative outcomes compared to the first-generation.

Discussion: The findings demonstrate meaningful advancements in the development of nitazene LFIA test strips, addressing critical analytical gaps posed by the first-generation test strips. The successful detection of desnitazenes suggests improved antibody recognition across structurally

divergent analogues, enhancing the utility of these devices in both forensic and harm reduction contexts. Resolution of caffeine cross-reactivity significantly improves result reliability, particularly given the prevalence of caffeine as a cutting agent in illicit drug samples. Nevertheless, variability in sensitivity across individual analogues persists, and the presumptive nature of LFIA testing necessitates confirmatory analysis using laboratory-based techniques. Ongoing surveillance of emerging nitazene analogues and iterative test strip development remains essential.

Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

Conflict of Interest

The authors declare that this project was funded by Innovate UK as part of a Knowledge Transfer Partnership with One-Use, the UK supplier and distributer of the evaluated test strips (manufactured by BTNX). Test strip materials were provided by the industry partner; however, the partner had no involvement in the study design, experimental work, data analysis, or interpretation of findings.

P-103

Conduct and Detect: A One-Step Conductometric Screen for Fraudulent Urine Specimens

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Abstract

Introduction: Urine sample tampering remains a significant challenge in forensic toxicology, especially in drug testing programs where individuals may attempt to evade detection. Manipulation strategies include dilution and the use of synthetic urine products designed to mimic physiological characteristics. Conventional validity testing relies primarily on creatinine and specific gravity; however, these parameters are often insufficient to differentiate between dilution mechanisms or to reliably identify synthetic matrices. Conductometry, represents a rapid, cost-effective analytical approach that may provide additional discriminatory power. This study evaluates the effectiveness of conductivity measurements in identifying various forms of urine sample adulteration.

Objectives: The primary objective of this study was to assess the utility of electrical conductivity as a screening tool for detecting fraudulent urine specimens. Specifically, we aimed to (i) determine whether conductivity can distinguish between authentic, diluted, and synthetic urine samples, (ii) evaluate its relationship with conventional validity parameters, and (iii) explore its ability to provide additional insight into the mechanisms of sample manipulation.

Methods: A total of 153 urine specimens were categorised into six groups: healthy volunteers (n=61), authentic drug-negative specimens(n=30), laboratory-prepared synthetic urine (n=5), commercial synthetic urine products(n=11), dilute specimens (n=30; defined by concurrent creatinine <20 mg/dL and specific gravity ≤1.002), and specimens flagged as fraudulent during routine forensic casework (n=16). For each sample, conductivity was measured at room temperature using a conductivity meter. Creatinine, pH, specific gravity and nitrite were measured using standard methods with CEDIA/DRI immunological test kits (Thermo Scientific). All test results were analysed using SPSS 25.

Results: The conductivity reference interval for healthy volunteers was 5.12–30.70 µS/cm (median 18.4 µS/cm). Strong positive correlations were observed between conductivity and both creatinine (ρ=0.675, $p<0.0001$) and specific gravity (ρ=0.708, $p<0.0001$). Authentic drug-negative specimens (median 21.4 µS/cm) were comparable to volunteer samples ($p=0.09$). All dilute specimens fell outside the reference interval, exhibiting a bimodal conductivity distribution: 8 specimens showed very low values (2.05–3.16 µS/cm), 22 showed markedly elevated values (997–1939 µS/cm). Critically, both subgroups were indistinguishable by conventional validity markers, median creatinine 13 mg/dL and specific gravity 1.002 in each, demonstrating that conductometry provides discriminatory information beyond standard testing parameters. Laboratory-prepared synthetic urine demonstrated consistently low conductivity values, significantly different from authentic samples. Commercial synthetic urine products showed heterogeneous profiles, with most falling within the reference range but some exhibiting abnormally high conductivity and nitrite positivity. Among forensic case samples, a subset displayed elevated conductivity despite unremarkable traditional parameters. Overall differences between groups were statistically significant ($p=0.0003$).

Discussion: Conductivity measurement identified as a highly sensitive and informative parameter for detecting urine sample tampering. Its strong correlation with established validity markers supports its physiological relevance, while its ability to detect all dilute samples highlights its diagnostic value. Importantly, the observed bimodal distribution among diluted specimens suggests that conductivity may provide insight into the underlying dilution mechanism, a feature not achievable with conventional tests alone. While laboratory-prepared synthetic urine was readily distinguishable, commercial products demonstrated variability, indicating ongoing challenges in universal detection. Overall, conductometry represents a practical, cost-effective addition to existing validity testing panels, offering enhanced detection capabilities in forensic toxicology laboratories.

Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

P-104

Validation of an Automated Extraction for the Quantitation and Identification of Cannabinoids in Oral Fluid for DUI Casework

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Abstract

Introduction: In 2018, the Alabama Department of Forensic Sciences (ADFS) implemented a statewide oral fluid drug testing program to support DUI investigations through roadside screening and laboratory confirmation. The ADFS Toxicology Discipline analyzes 13 cannabinoids in oral fluid specimens, with 4 cannabinoids—delta 9 tetrahydrocannabinol (THC), carboxy-THC, hydroxy-THC, and cannabidiol—quantitatively evaluated. The current extraction procedure utilizes manual liquid-liquid extraction (LLE). To enhance extraction efficiency, ADFS validated an automated extraction procedure for cannabinoids in oral fluid specimens.

Objectives: To validate an automated extraction for the quantitation and identification of cannabinoids in oral fluid specimens for use in DUI casework.

Methods: Oral fluid specimens were extracted via solid-phase extraction (SPE) using Dispersive Pipette XTRaction (DPX) Supel Swift HLB (hydrophilic and lipophilic balanced) tips on a Hamilton MICROLAB STARlet. The DPX tips were conditioned with methanol and equilibrated with water prior to sample loading. Within the DPX tips, samples were washed with water followed by 30:70 methanol:water. Target analytes were eluted into a well plate via 50:50 methanol:acetonitrile and evaporated to dryness at 40°C using Hamilton's Multi-Flow Positive Pressure Evaporation Extraction (MPE^2) Module. Dry samples were reconstituted in a mixture of 50% mobile phase A—water with 5mM ammonium formate with 0.1% formic acid–and 50% mobile phase B—methanol with 0.1% formic acid. Samples were transferred to autosampler vials for analysis via liquid chromatography-tandem mass spectrometry (LC/MS/MS).

An automation specific study—channel carryover—was conducted to ensure contamination was not occurring during the automated extraction process. Additional validation studies include accuracy, precision, limit of detection, interferences, stability, previously analyzed casework, and dilution integrity.

Results: Preliminary studies were performed to determine if cross-contamination was occurring between adjacent wells within a 96-well plate during the automated extraction, known as channel carryover. Analysis of samples revealed contamination occurring between plate columns, though no obvious sources of contamination were observed. The SPE steps were modified to occur sequentially across the columns of a single well plate, eliminating the risk of contamination between samples.

The automated extraction maintained the linear calibration model validated for the manual extraction, while the re-validation process was used to expand the upper limit of quantitation from 300 ng/mL to 500 ng/mL without sacrificing accuracy and precision. Limits of detection validated for the manual extraction were achieved by the automated extraction. Minimal matrix and analyte interferences were observed. Delta 9 THC, carboxy-THC, and cannabidiol maintained appropriate accuracy when in-vial dilutions were performed at dilution factors of 2X, 5X, and 10X. The analysis of previously analyzed casework and simulated casework samples demonstrated the automated extraction method provided accurate results.

Discussion: Validation of the automated extraction method for cannabinoids in oral fluid confirmed its suitability as an alternative for the manual procedure. While the method is functional, user interaction is often required to resolve errors caused by minor shifts in plate positions. Advanced programming modifications are being researched to optimize the automation process, aiming to minimize user interaction and enable scientists to focus on other essential tasks.

Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

P-105

Into the Unknown: Evaluating Time of Flight Drug Screening Against Gas Chromatography Mass Spectrometry Drug Screening

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Abstract

Introduction: The ability to screen and detect a broad range of drugs remains an ongoing challenge in toxicology, specifically finding the balance between time, cost, and efficiency. Gas chromatography mass spectrometry (GC/MS) has been the standard screening method in many laboratories for several decades; however, the requirement for high sample volume and reduced sensitivity have driven the development and transition to new extraction methods and technology. Liquid chromatography-quadrupole time-of-flight mass spectrometry (TOF) enables a more sensitive and expanded scope of drug detection.

Objectives: To compare the results from cases analyzed using both the GC/MS and TOF screening methods with emphasis on concordant results and targets not detected by one or both methods. Concordant results and targets uniquely detected by each method were evaluated to determine whether the TOF screening method was equivalent or better than historical GC/MS basic drug screen (BDS).

Methods: The BDS method consists of a liquid-liquid extraction followed by GC/MS analysis using an Agilent 8890 GC System with 5977B GC/MSD, with data processed through a combination of Mass Hunter Unknowns and ChemStation Analysis. Drug extraction for TOF screening used a Thomson filtration extraction with analysis utilizing the 1290 Infinity II and 6545 Q-TOF Liquid Chromatography Mass Spectrometry (LC/MS); data is analyzed using Mass Hunter Qualitative Analysis. Cases containing analyses by both the TOF screen and GCMS were isolated and evaluated. The level of agreement between GC/MS and TOF results was assessed.

Results: A total of 596 cases analyzed March 2024 through April 2026 were compared. In this data set, more than 2,200 targets were detected. In 2% of cases, no drugs were detected by either method. In 21% of cases, both methods were in agreement, whereas in 79% at least one identified target was not detected by both methods. Of these discordant results, 6% were detected only by the BDS method, while 94% were detected only by TOF.

Discussion: Of the targets detected only by TOF, 9% were not within the validated scope of the BDS method and are more commonly identified using the acidic/neutral drug screen (ANS), which was not evaluated in this study. Many targets detected only by GC/MS were metabolites not currently in the TOF database; whereas targets detected only by TOF were often present at concentrations below the GC/MS limit of detection. TOF provides a broader range of detectable targets and improved sensitivity compared to the GC/MS method. Additionally, the TOF extraction method requires less specimen volume with less solvent waste. Comparison of these methods will continue to be monitored with further evaluation including ANS results. The TOF-based general unknown screening approach has demonstrated fit-for-purpose performance as a primary screening method with expanded analytical scope compared to the current GC/MS BDS.

Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

P-106

Evaluation of Colorimetric, Microcrystalline, and LC-MS/MS Methods for Emerging Synthetic Drug Adulterants: Addressing Specificity Gaps in Modern Drug Detection

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Abstract

Introduction: Reliable detection methods are essential for identifying controlled drugs in complex, adulterated samples. Forensic labs employ a range of analytical techniques (color/spot tests, microcrystalline tests, SEM, and LC–MS/MS) to secure exact identification and confirmation. In public harm-reduction settings, rapid immunoassay-based tools are increasingly used for on-site screening, and drug-detection kits are widely used by law enforcement for preliminary field testing. However, cross-reactivity with common household materials and over-the-counter pharmaceuticals challenges reliable detection. This study evaluates the effectiveness, specificity, and cross-reactivity of these techniques, establishing a complete framework for rapid, reliable drug detection supporting forensic investigations and harm-reduction efforts.

Objectives: To evaluate the effectiveness, specificity, and cross-reactivity of a panel of chemical spot tests, microcrystalline tests, immunoassays, on-site screening tools, and LC–MS/MS for detecting emerging adulterants; and to identify reliable reagents and assess false positive risk, including cross-reactivity with other drugs, household materials, and OTC substances.

Methods: A broad panel of spot test and microcrystalline reagents was screened to identify those producing specific, distinguishable color changes and microcrystals for xylazine, BTMPS, and combinations of fentanyl, xylazine, and BTMPS. An LC-MS/MS method was developed and validated for the selective detection and confirmation of these analytes in urine, including blind testing and performance evaluation under simulated forensic conditions. The reliability of multiple commercially available xylazine-specific immunoassay products and on-site law enforcement field test kits was assessed against xylazine, BTMPS, a range of controlled and non-controlled substances, and common household and OTC materials to evaluate specificity and cross-reactivity.

Results: Eight spot test reagents yielded distinct color transitions, including cobalt thiocyanate, Eosin Y (pH 7.0), Liebermann, Mandelin, Mecke, Marquis, Scott, and Young. Gold chloride microcrystalline testing yielded unique microcrystalline structures, further characterized by SEM. The LC–MS/MS method enabled selective identification and quantitation of xylazine and BTMPS in urine, extending the validated detection to a biological matrix not previously addressed. The majority of xylazine immunoassay products reliably detected the target compound; however, lidocaine and BTMPS were identified as cross-reactants producing false positives with select strips. A large number of on-site drug screening kits exhibited false positives due to non-specific functional-group interactions, and evaluation of presumptive field test kits against household and OTC substances showed consistent cross-reactivity across multiple reagents.

Discussion: Chemical spot tests, microcrystalline tests, immunoassays, on-site screening tools, and LC–MS/MS vary in their success in detecting and identifying controlled drugs. While presumptive methods enable rapid screening, their use on functional-group reactivity results in limited specificity and a high risk of false positives, particularly with emerging adulterants such

as xylazine and BTMPS. An evaluation of field drug test kits highlights limitations when used independently. These findings stress that independent use of presumptive tools risks widespread misclassification; it is therefore imperative to establish and validate new detection protocols for emerging substances and integrate confirmatory instrumentation to address the analytical challenges presented by the modern illicit drug landscape.

Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

P-107

Comparison of Enzymatic Hydrolysis and Formic Acid Acidification for Improved Detection of Drug Metabolites in Non-Fatal Overdose Urine by LC-QToF-MS

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Abstract

Introduction: Non-fatal overdose (NFOD) cases represent a critical but under-studied aspect of the opioid epidemic and provide a unique opportunity to examine drug exposure and metabolism in living individuals. Drug detection is complicated by phase II metabolism, which produces glucuronide and sulfate conjugates that are often poorly detected without hydrolysis. These conjugated metabolites represent major excretion products and provide important information regarding drug exposure and biotransformation pathways, making accurate characterization essential for toxicological assessment. Urine exhibits substantial variability in pH, hydration, and metabolite excretion, all of which can influence hydrolysis efficiency and analyte ionization. Laboratories may use enzymatic hydrolysis or acidification to address these challenges. Enzymatic hydrolysis generally provides more complete glucuronide cleavage under mild conditions, whereas acidification primarily enhances mass spectrometry (MS) signal intensity. These differences highlight the need to evaluate sample preparation strategies for improved metabolite detection.

Objectives: The primary objective was to qualitatively evaluate differences in drug metabolite detection and signal consistency in non-fatal opioid overdose urine samples by comparing B-One All-in-One β-glucuronidase (Kura Biotech) enzymatic hydrolysis with chemical acidification using liquid chromatography-quadrupole time-of-flight mass spectrometry (LC-QToF-MS).

Methods: A targeted panel of 12 drug glucuronide standards was evaluated, including opioid glucuronides (morphine-3-, morphine-6-, codeine-6-, hydromorphone-3-, oxymorphone-3-, buprenorphine, norbuprenorphine, and naloxone-3-glucuronide), benzodiazepine glucuronides (lorazepam, oxazepam, and temazepam), and THC-COOH glucuronide. Pooled human urine was fortified with glucuronide standards at 25, 75, 250, and 500 ng/mL for all analytes except naloxone glucuronide (50, 500, and 1000 ng/mL). Samples underwent one of three preparation conditions: (1) acidification only, (2) acidification followed by enzymatic hydrolysis, or (3) enzymatic hydrolysis followed by acidification. Acidification with 0.1% formic acid in methanol (1:1, v/v) and treatment with 50 µL B-One All-in-One β-glucuronidase were performed in the indicated order, followed by a 15-minute room-temperature incubation. Glucuronide-spiked samples and authentic NFOD urine specimens (n = 9*) were processed using each preparation condition. Following methanol quenching, samples were analyzed by LC-QToF-MS and compared against an in-house spectral library containing hundreds of psychoactive drugs and metabolites to assess the effects of treatment sequence on glucuronide deconjugation and analyte detection.

Results: In fortified pooled urine, samples prepared with 0.1% formic acid alone produced lower parent-compound signals, greater analytical variability, and fewer detectable deconjugated analytes, consistent with limited glucuronide cleavage under these conditions. In contrast, enzymatic hydrolysis with B-One All-in-One β-glucuronidase yielded a greater number of detectable deconjugated parent compounds and consistently higher hydrolysis efficiencies, defined as the response of liberated parent compound relative to glucuronide-derived signal or

pre-hydrolysis baseline. Signal variability was also reduced, indicating improved reproducibility. Combining enzymatic hydrolysis followed by acidification further increased signal intensity for several analytes, consistent with enhanced ionization under normalized acidic pH conditions.

Discussion: B-One enzymatic hydrolysis followed by acidification with 0.1% formic acid improved hydrolysis efficiency and signal consistency compared with acidification alone. This approach enhanced detection of glucuronide-derived metabolites while reducing variability associated with incomplete hydrolysis and matrix effects. These findings support enzymatic hydrolysis as a reliable approach for LC-QToF-MS analysis of glucuronide metabolites in urine, improving the consistency and interpretability of toxicological measurements in non-fatal opioid overdose investigations.

Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

P-108

Development of Conditions for HPLC Chromatography of the Substance Dimetindene Maleate and Its Toxic Chemical Degradation Products

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Abstract

Introduction: The State Pharmacopoeia of Ukraine (SPU) does not regulate the analysis of the substance dimetinden maleate. The analysis of the substance is regulated by monograph 01/2022:1417 of the European Pharmacopoeia (Eur.Pharm.). The task is to modernize the analysis of the substance, develop or modify research methods using a more effective high-performance liquid chromatography (HPLC) method, which will allow expanding the range of instrumental methods for studying the substance and offering new research methods.

Objectives: HPLC identification of the substance dimetindene maleate and toxic degradation products of its molecules using standardized validated methods and research conditions that were previously developed for analysis by gas chromatography (GC) according to Eur.Pharm. with appropriate modifications.

Methods: HPLC, Agilent 1200 chromatograph, DAD detector, column – ACE Excel 3 Super C18, 100×4.6 mm; computer analysis by OpenLab CDS program. Reagents (HPLC grade): ammonium formate, formic acid, methanol, acetic acid, sodium hydroxide. Composition of mobile phases: A – 950 ml of buffer solution A, 50 ml of methanol P; B – 50 ml of buffer solution A, 950 ml of methanol P. The buffer solutions: A – 3.25 g of ammonium formate in 1000 ml of water P (pH 3.5 is obtained using formic acid), B – 3.25 g of ammonium formate in 50 ml of water P (pH 3.5 is obtained using formic acid).

Results: The conditions for chromatographic analysis of the research objects and the research methodology were subject to modification. In order to implement the HPLC method in the analysis of a substance, we proposed the composition of the mobile phases (A and B), of buffer solutions (A and B). Detection was performed with a DAD at 258 nm. The column temperature was reduced from 40°C to 35°C.The use of HPLC was successful because this method has certain advantages compared to GC: speed of analysis, high identification ability, accuracy of the results obtained.

Discussion: Performs impurity control in substances for pharmaceutical use: A, B, C, D, E, F, G, H, I. For example, Impurity A - 2-Ethylpyridine is a toxic substance (skin, eye, respiratory irritation). The toxicity of it was predicted by program Protox 3.0. Impurity A has an LD50 2280 mg/kg (class 5), predicted biotargets of toxic effects are Histamine H1 receptors, 80% of known related ligands. It exhibits neurotoxicity, ecotoxicity, and BBB-barrier toxicity. Toxic Impurity A was not detected in the substance by HPLC. However, in the chromatograms we observe clear analytical signals of maleic acid and benzoic acid (maleic acid Rt=1.286 min, benzoic acid Rt=7.182 min). Maleic acid belongs to unspecified impurities and is possibly a product of chemical degradation of the substance dimetindene maleate. Benzoic acid belongs to low-toxic substances (class 4, GHS/CLP). Maleic acid is more toxic than benzoic acid and is an unacceptable impurity in the composition of the test substance. The HPLC has been successfully implemented in the analysis, which allows it to

be considered an alternative to the GC. Not all detected impurities can be identified by Rt, further analysis requires the use of mass spectrometry or combination of methods.

Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

P-109

A Rapid and Highly Sensitive Analytical Method for Comprehensive Determination of Cocaine and Hydroxy Metabolites in Hair

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Abstract

Introduction: Cocaine, a central nervous system stimulant with a high potential for abuse and severe psychological and physical dependence, is often encountered in forensic and clinical cases. Hair is a complementary biological matrix that provides an extended detection window (months), offering insights beyond blood and urine tests, especially in drug-facilitated crimes, child custody cases, and workplace drug testing. However, hair testing faces the challenge of external contamination. Previous research recommended detecting hydroxylated cocaine metabolites to confirm cocaine use. Most of these methods focus on limited metabolite panels and/or require a large sample size.

Objectives: This study aimed to develop and validate a rapid, simple, and sensitive analytical method in hair for cocaine and ten metabolites, benzoylecgonine (BE), anhydroecgonine methyl ester (AEME), ecgonine methyl ester (EME), norcocaine (NCOC), cocaethylene (CE), two hydroxy-benzoylecgonine isomers (m-OH-BE, p-OH-BE) and three hydroxy-cocaine isomers (m-OH-COC, p-OH-COC, and o-OH-COC).

Methods: The method was optimized using authentic cocaine-user hair samples, evaluating snippets/hair powder, different hair amount (5-10mg), and incubation procedures (bead mill, ultrasound, time, temperature). Samples were donated by the Center for Forensic Hair Analytics (University of Zurich, Switzerland). The optimized procedure consisted of 5 mg finely cut hair and 25µL of internal standard at 0.1µg/mL (COC-d_3, BE- d_3, CE- d_3, NCOC-d_3, EME-d_3, p-OH-COC-d_3, and m-OH-BE-d_3), simultaneously homogenized and incubated in 1mL methanol in a bead mill (five 1-minute cycles, speed 6 min/s, dwell time 5 minutes). Twenty-five µL 1% HCl:MeOH solution was added to the extract before evaporation, reconstituted in 200µL of 10 mM ammonium formate in 0.1% formic acid in water: 0.1% formic acid in acetonitrile (98:2), and analyzed by liquid chromatography-tandem mass spectrometry. The chromatographic separation used a reversed-phase column in gradient mode, using 10 mM ammonium formate in 0.1% formic acid in water and 0.1% formic acid in acetonitrile as mobile phases. Two MRM transitions per compound were monitored using electrospray in positive mode.

Results: The method showed good linearity, 0.05-5ng/mg for COC and 0.01-1ng/mg for BE, CE, NCOC, AEME, EME, m-OH-COC, o-OH-COC, p-OH-COC, m-OH-BE, and p-OH-BE. Imprecision at low (0.03/0.15ng/mg), medium (0.3ng/mg), and high (0.7ng/mg) QC (n=15) were <19.8% (n=15) and bias from -11.8 to 4.7% (n=15). No matrix effect (n=10, CV<25%) was detected for COC, BE, NCOC, m-OH-COC, p-OH-COC, m-OH-BE, and p-OH-BE. However, CE, AEME, EME, and o-OH-COC showed ion suppression (-28.6 to 46%). Extraction efficiency ranged from 70 to 81%, and process efficiency ranged from 54 to 107%. Extracts were stable in the autosampler (10°C, 24h), and no exogenous interferences or carryover were observed after a hair sample at 5 ng/mg.

Discussion: A fast, sensitive, and specific method for determining cocaine and 10 metabolites, including the hydroxy-COC and hydroxy-BE metabolites, in hair was developed and validated. Using 5 mg of hair, the method achieved limits of quantification from 0.01 to 0.05 ng/mg, depending on the analyte, fulfilling and improving the Society of Hair Testing Consensus on Drugs of Abuse requirements (cut-off for cocaine of 0.5 ng/mg hair, and the presence of benzoylecgonine, norcocaine, cocaethylene, hydroxyl-cocaines or hydroxy benzoylecgonine).

Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

P-110

A QuEChERS LC/MS/MS Comprehensive Method for Abuse Drugs, Medications, and Pesticide Detection on Biological Samples in the Toxicology Unit of the Costa Rican Forensic Science Laboratory Department

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Abstract

Introduction: In modern times, with the large number of drugs of abuse, new psychoactive substances, and different groups of medications, forensic toxicology laboratories face the challenge of developing analytically valid methods for the simultaneous detection of as many substances as possible.

Objectives:

1. Develop a screening qualitative method that allows the simultaneous detection of different groups of drugs of abuse, medications, new psychoactive substances and pesticides in forensic biological origin samples.
2. Determine the analytical performance parameters of the developed method, by applying the Standard Practices for Method Validation in Forensic Toxicology, ANSI/ASB Standard 036, First Edition, 2019.
3. Use the validated method for the toxicological analysis of samples received from various types of cases where the Judicial Authority requires it.

Methods: For the determination, deuterated internal standards for each familly of substances were used and certified reference material for each of the analytes of interest, were spiked on the positive controls. Subsequently, the samples are processed using the QuEChERS (AOAC) extraction method. Instrumental analysis of the extracts is performed using an LC/MS HRMS equipment.

For blood samples, testing requires a minimum volume of 0.5 mL, and collection with potassium oxalate anticoagulant and sodium fluoride preservative. For urine samples, a minimum of 0.5 mL of matrix should be used, collected and stored in plastic tubes and preserved with sodium fluoride. For vitreous humor, at least 200 µL is required. For viscera, at least 1 g of sample is required, and for gastric contents, a minimum of 0.2 g of sample.

Results: Once validation was completed, acceptable results were obtained for LOD (benzodiazepines:10-40 ng/mL, antidepressants: 25-50 ng/mL, fentanile: 5ng/mL, other opiates and opioids: 25-50 ng/mL, amphetamines: 30-50 ng/mL, antipsychotics: 25-50 ng/mL), stability (more than 24 hours), matrix Ionization Suppression/Enhancement (less than 25% effect on postmortem blood, urine and regular blood for most of the analytes), carryover (No signal above the Detection Limit were present on mobile phase blank after injection of an analyte above the ULQ). Finally, the method has accomplished satisfactory results in identification parameters such as: retention time (coeffcient of variation less than 2.5%) and full spectrum library comparison (similarity index greater than 800) for the substances included in the method.

Discussion: The development of comprehensive methodologies for the simultaneous detection of different families of analytes is of particular interest and relevance in the forensic toxicology area, where laboratories must deal with limited amounts of evidence, complex analytical matrices and budget limitations. Currently, this methodology is used in casework to analyze drug-facilitated crimes, postmortem cases of intentional or accidental poisoning, malpractices, cases of child deaths or deaths while in the custody of authorities. It allows for the simultaneous detection of more than 100 substances with simple extraction procedure on different kinds of matrices such as gastric contents, blood, urine, viscera, and vitreous humor.

Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

P-111

Molecular Mirrors: Enantioselective LC–MS/MS Differentiation of Amphetamine and Methamphetamine in Whole Blood

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Abstract

Introduction: Amphetamine and methamphetamine are central nervous system stimulants used as recreational drugs but also as therapeutic options in conditions such as ADHD or narcolepsy, potentially complicating the forensic interpretation of toxicological findings. Amphetamine may also arise as a metabolite of prescribed medications such as selegiline and lisdexamfetamine.

Both compounds are chiral molecules with a single stereogenic center and therefore exist as dextrorotatory (D-) and levorotatory (L-) enantiomers. The D-enantiomers are primarily responsible for central stimulant effects, whereas L-enantiomers are mainly associated with peripheral activity and may be present in certain pharmaceutical or over-the-counter formulations. As exposure may involve single enantiomers or racemic mixtures, enantiomeric differentiation provides valuable information for result interpretation and becomes increasingly important in differentiating between therapeutic and recreational use; however, conventional toxicological analytical methods typically do not distinguish between them.

Objectives: This study aimed to develop and validate a direct LC–MS/MS method for the enantioselective determination of amphetamine and methamphetamine in whole blood and to evaluate its applicability in forensic casework.

Methods: Whole blood samples underwent protein precipitation, phospholipid removal, evaporation, and reconstitution in methanol prior to analysis. Enantioselective chromatographic separation was achieved using a Lux® 3 µm AMP chiral column (Phenomenex) under optimized conditions. Baseline enantiomeric separation was obtained for amphetamine, while satisfactory resolution allowing reliable quantification was achieved for methamphetamine.

Results: The method validation was done according to ANSI/ASB Standard 036 guidelines, including assessment of linearity, limits of detection and quantification, precision, accuracy, selectivity, carryover, and matrix effects. The method demonstrated adequate sensitivity, selectivity, and robustness for forensic application, with precision and accuracy within ±20%.

The method was successfully applied to eleven authentic forensic cases encompassing distinct enantiomeric profiles. Eight cases showed exclusively D-amphetamine, including one with documented therapeutic use of lisdexamfetamine (Elvanse®), indicating legal prescription drug consumption. One case presented both D- and L-amphetamine (154 ng/mL and 375 ng/mL respectively), suggesting intake of illegal racemic amphetamine. The non-equimolar ratio likely stems from the slightly shorter elimination half-life of d-amphetamine compared to l-amphetamine. In another case, L-amphetamine and L-methamphetamine (2,9 ng/mL and 5,7 ng/mL respectively) were detected, a profile consistent with certain pharmaceutical or over-the-counter products.

Discussion: This validated LC–MS/MS method enables reliable enantiomeric differentiation of amphetamine-type stimulants in whole blood and strengthens forensic interpretation by supporting the distinction between illicit use and therapeutic administration based on enantiomeric profiles.

Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

P-112

LC-MS/MS Methods for Alcohol Biomarkers in Urine and Whole Blood

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Abstract

Introduction: Several established biomarkers are available for monitoring prior alcohol consumption in biological samples using LC-MS/MS. Selection of the appropriate biomarker depends on clinical objectives, including detection of recent alcohol use (within ~3 days) or assessment of longer-term consumption (2–4 weeks). This study presents two analytical methods, one for quantifying ethyl glucuronide (EtG) and ethyl sulfate (EtS) in urine, and another for the analysis of phosphatidylethanol (PEth) in whole blood, providing complementary tools for alcohol monitoring.

Objectives: To develop and demonstrate two LC-MS/MS workflows for alcohol metabolite biomarkers in biological matrices: one workflow for quantifying short-term alcohol use biomarkers (EtG and EtS) in urine and another method for assessing drinking patterns through PEth analysis in whole blood.

Methods: Analyses were performed using a Shimadzu LC-MS 8060 triple quadrupole mass spectrometer operating in ESI negative mode. For urine analysis, nine EtG and EtS calibrators (50–2,000 ng/mL) were prepared in synthetic urine. Quality control samples were generated at 100, 400, and 1,600 ng/mL using six unique lots of analyte-free human urine, including two kidney disease patients to evaluate samples with enhanced potential for matrix effects. Samples were diluted 20-fold with water containing 0.1% formic acid, vortexed, centrifuged, and injected (10 µL). Chromatographic separation was achieved using a Force Biphenyl column (100 × 3 mm, 3 µm) with water and methanol (both containing 0.1% formic acid) as mobile phases. The flow rate was 0.8 mL/min, the column oven was set to 30 °C, and the overall run time was 5-minutes utilizing gradient conditions.

For whole blood analysis, nine PEth homologue 16:0/18:1 and 16:0/18:2 calibrators were prepared in bovine whole blood at a range of 10-1,000 ng/mL, with quality controls prepared at 15, 100, and 500 ng/mL in analyte-free human whole blood. Samples (50 µL) were extracted with 150 µL of 4:1 isopropanol:tetrahydrofuran, vortexed, centrifuged, and injected (5 µL). Separation was performed using a Raptor C8 column (50 × 2.1 mm, 2.7 µm) with mobile phases consisting of water with 5 mM ammonium acetate and acetonitrile/isopropanol (90:10). The flow rate was 0.6 mL/min, the column oven was set to 30 °C, and the overall run time was 5-minutes using gradient conditions.

Results: Both methods demonstrated excellent linearity, with correlation coefficients (r^2) ≥ 0.995. Calibration for EtG and EtS utilized 1/x-weighted quadratic regression, whereas PEth was calibrated using 1/x-weighted linear regression. Intraday precision and accuracy for EtG and EtS showed percent recoveries ranging from 85.1-114.6%, with %RSD values ≤9.6%. For PEth, percent recoveries were within 10% of the nominal concentration, with %RSD values ≤14.2%. Interday performance for both methods showed percent recoveries within 11.9% of the nominal range, with %RSD values ≤10.1%.

Discussion: Two robust LC-MS/MS methods were successfully developed for the quantification of alcohol biomarkers in urine and whole blood. Both workflows are rapid, sensitive, and reliable, meeting the performance requirements for clinical and forensic applications, and enabling flexible monitoring of both short- and long-term alcohol consumption.

Disclosure

Yes, I, or a member of my immediate family, has a financial interest to disclose.

Conflict of Interest

Salary

P-113

Comparative Evaluation of High-Resolution Mass Spectrometry Acquisition Strategies for the Detection and Analysis of Illicit Drug Substances

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Abstract

Introduction: Drug screening is a critical component of successful drug treatment programs worldwide, supporting both compliance monitoring and the detection of illicit substance use. Traditionally, laboratory workflows rely on an initial immunoassay screen followed by confirmatory analysis using LC-MS/MS. More recently, some service providers have streamlined this process by adopting a single targeted LC-MS/MS method, focused on a select group of key analytes. Although both approaches are effective for limited analyte panels, they lack the breadth needed to detect a wider range of drug substances.

Objectives: This study investigates the performance of various time-of-flight (Tof) acquisition strategies and assesses their ability to meet existing service requirements while expanding analytical capability to enable broad, comprehensive drug screening using the same instrumentation.

Methods: Quantitative analysis was carried out for 20 drug substances, including benzoylecgonine, etonitazene, ketamine, methamphetamine, mitragynine, orphines, opioids and temazepam, using the benchtop hybrid quadrupole multi-reflecting time-of-flight mass spectrometer (MS), the Xevo™ MRT MS (Waters™). Data were acquired using both Tof-MRM (data-dependent type) and Tof-MS^E (data-independent type) acquisition modes. Pooled blank urine samples were fortified with the target analytes across a concentration range of 0.01 to 2500 ng/mL, followed by a simple 1:5 dilution step prior to LC-MS analysis. Data were additionally collected for the commercial reference material, the Waters system suitability mixture at 25 ng/mL, alongside 20 anonymized authentic urine samples (diluted 1:5). Tof-MS^E data were acquired using the Waters Forensic Toxicology Screening solution, in ESI+ ionization mode, with a collision energy ramp of 10-40eV. Chromatographic separation was achieved using a reversed phase C_{18} column, comprising a 15-minute gradient elution, maintained at 50°C. For Tof-MRM mode, data were acquired using the same chromatographic conditions only. For each analyte, two exact-mass product ions (a quantifier and a qualifier) were monitored, with cone voltages and collision energies individually optimized.

Results: In this investigation, the Tof-MRM acquisition mode demonstrated a marked enhancement in analytical sensitivity, achieving limits of detection (LOD) of 0.5 ng/mL (or lower) for all compounds, compared with Tof-MS^E (5 ng/mL or lower). This superior performance emphasizes the sensitivity and robustness of the Tof-MRM approach, highlighting its capacity to reliably quantify analytes with greater precision and confidence on a high-resolution mass spectrometer. The methods were subsequently applied to a set of authentic urine samples (n=20), and the results showed strong quantitative agreement. Importantly, the Tof-MS^E acquisition strategy enabled the identification of additional substances outside the predefined targeted panel, encompassing frequently encountered medications, benzodiazepines, antidepressants, and illicit drug substances, which would have otherwise gone unobserved.

Discussion: Both data-dependent and data-independent Tof acquisition modes, achieving LOD values of 0.5 ng/mL and 5 ng/mL or lower, respectively, demonstrated a simple, robust, and highly sensitive analytical approach for urinary drug quantification, delivering sensitivity comparable to conventional tandem-quadrupole methodologies. Furthermore, incorporation of the complementary Tof-MS^E acquisition, further enhanced overall sample characterization, enabling the identification of additional drug substances beyond the predefined targeted panel. Together, these strategies provide a powerful and comprehensive framework for drug screening and quantitation in complex biological matrices.

Disclosure

Yes, I, or a member of my immediate family, has a financial interest to disclose.

Conflict of Interest

I am a paid employee of Waters Corporation

P-114

A Ready-to-Run Workflow for Quantitating 80 Drugs of Abuse in Whole Blood with the TSQ Certis Triple Quadrupole MS

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Abstract

Introduction: The accurate and reliable quantitation of drugs of abuse in whole blood is critical for forensic toxicology and clinical research investigations. Analytical laboratories face ongoing challenges in meeting increasing demands for high-throughput, sensitive, and reproducible testing while maintaining compliance with regulatory standards. This workflow presents an end-to-end solution for the quantitation of 80 commonly encountered drugs of abuse in whole blood using the TSQ Certis triple quadrupole mass spectrometer. The workflow integrates efficient sample preparation, robust chromatographic separation, and optimized mass spectrometric detection to deliver exceptional sensitivity, selectivity, and quantitative accuracy.

Objectives: To show a quantitative workflow for 80 drugs of abuse in whole blood featuring polarity switching and a 4.5-minute analysis equaling 320 samples per day on the TSQ Certis mass spectrometer.

Highlight a streamlined sample preparation with efficient protein precipitation and filtration using INTip™ Filtration (DPX Technologies).

Methods: A total of 80 target analytes were prepared into a mix to be spiked into negative human whole blood. Calibration standards were then prepared at 13 levels ranging from 0.05 to 500 ng/mL in whole blood to a total volume of 100 µL (blood + sike solution). Samples were spiked with 15 µL of internal standard (ISTD) stock comprised of the labeled standards of each of the 80 drugs. A protein crash was performed with 25 µL of 1% Zinc Sulfate (ZnSO4) and 300 µL of acetonitrile. Samples then underwent INTip™ Filtration using Tip-on-Tip™ (ToT) technology from DPX Technologies. Samples were dried down and reconstituted with a 20:80 mixture of MeOH with 0.1% formic acid and 2 mM ammonium formate and H2O with 0.1% formic acid and 2 mM ammonium formate.

Data was acquired on the TSQ Certis mass spectrometer using SRM mode for a quantitative and a confirming transition for each target compound and a quantitative transition for the internal standards. Q1 resolution was 0.7 FWHM and Q3 1.2 FWHM. The SRM list contained the retention times, precursor and product ions m/z, optimized RF lens voltages, and collision energies.

Data was acquired and processed with Thermo Scientific™ TraceFinder™ software, version 5.2.

Results: This workflow helped achieve LOQs as low as 0.05 ng/mL with ULOLs up to 500 ng/mL. Criteria for LOQ included %CV and %RSD being less than 20%. Calibration curves R^2 were greater than 0.9900. The TSQ Certis mass spectrometer uses the Thermo Scientific™ OptaMax™ Plus HESI source which allows for increased vaporizer temperatures, improving the relative peak areas and overall sensitivity for drugs of abuse. Utilizing a vaporizer temperature of 500˚C helped achieve improved sensitivity. A robustness study was performed by injecting extracted whole blood spiked with drugs of abuse for over 450 injections. Peaks areas were monitored from injection to injection. Lorazepam peak areas over time were well within a +/-20% RSD.

Discussion: This fast, quantitative TSQ Certis mass spectrometry method was used to detect and confirm 80 drugs of abuse and their corresponding internal standards, including positive and negative polarities, in whole blood in a 4.5-minute method.

Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

P-115

Validation of the Neogen® Gabapentin ELISA Kit for Whole Blood and Urine Specimens

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Abstract

Introduction: Gabapentin is an anticonvulsant medication approved by the FDA to treat epilepsy and postherpetic neuralgia and is widely prescribed off-label for a variety of ailments. The rising prevalence of gabapentin in impaired driving cases led the National Safety Council Alcohol, Drugs, and Impairment Division to elevate it from a Tier II to a Tier I compound in their 2025 recommendations for routine drug testing. As such, it is important for laboratories to develop robust testing methods for gabapentin.

Objectives: To validate a method for the use of the Neogen® Gabapentin Forensic Kit to screen for gabapentin in whole blood and urine specimens by enzyme-linked immunoassay (ELISA).

Methods: A Dynex DSX Automated ELISA System with the Neogen® Gabapentin Forensic Kit was used for this validation. A 1,000 ng/mL decision point was validated for both whole blood and urine specimens. Kit instructions were followed with the exception that the plate reader shake was decreased to 5 seconds. The required amount of concentrated gabapentin conjugate was diluted 1:100 with gabapentin conjugate diluent the day of analysis. Method validation was performed based on ANSI/ASB Standard 036 and in-house validation requirements. Sensitivity, carryover, hook effects, plate drift, precision, specificity, cross reactivity, robustness, and previously characterized authentic cases were evaluated.

Results: The theoretical limit of detection (LOD) was determined to be 346 ng/mL in blood and 186 ng/mL in urine. The experimental LOD was determined to be at least 500 ng/mL in both blood and urine as the assay could not reliably distinguish between 250 ng/mL and 500 ng/mL. No carryover or hook effects were observed at 100 µg/mL in whole blood and 10 mg/mL in urine. ANSI/ASB criteria were met in precision experiments and the CV of 15 replicates of gabapentin at concentrations of 0, 500, 1,000, and 2,000 ng/mL was no greater than 11.4% for whole blood and 12.1% for urine. Plate drift was evaluated using twenty-four intra-run replicates of gabapentin at the cutoff level and no statistically significant plate drift was detected. One data point in blood was determined to be an outlier using Grubb's test and was excluded from statistical calculations. Cross reactivity was initially evaluated for gabapentin lactam (< 5%) and pregabalin (1%). Authentic specimens, 19 urine and 13 blood, that contained 69 and 65 different substances in urine and blood, respectively, were tested for a crossover study. A false positive was observed for a urine case that had overloaded responses for benzoylecgonine and levamisole by other testing methods. This finding prompted further study of benzoylecgonine and levamisole at high concentrations for cross reactivity with the gabapentin assay. Benzoylecgonine did not demonstrate any significant cross reactivity (< 0.1%). It was verified that a concentration of 50 µg/mL of levamisole in urine, which had a cross reactivity of 2.3% in blood and 2.7% in urine, could cause a positive response.

Discussion: The Neogen® Gabapentin Forensic Kit demonstrated excellent precision and appropriate sensitivity, making it an efficient and reliable method to screen whole blood and urine specimens for gabapentin.

Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

P-116

LC–MS/MS Analysis of Caffeine in Electronic Cigarettes and its Pharmacokinetic Application to Biological Samples

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Abstract

Introduction: Caffeine (Caf) is widely consumed in beverages and supplements and has recently been incorporated into electronic cigarette (e-cigarette) products. The pharmacological effects and safety of Caf following oral administration are well established. However, available information on the inhalation of vaporized Caf via e-cigarettes remains limited. In particular, the health effects, including potential addiction associated with inhalation of vaporized Caf, are not fully clarified. In addition, exposure to unknown compounds generated during the heating process may pose further health risks. Therefore, comprehensive evaluations of chemical composition in smoke and pharmacokinetics following exposure and inhalation of vaporized Caf are required.

Objectives: This study aimed to analyze cigarette smoke extract (CSE) using the validated LC-MS/MS method. In addition, the pharmacokinetics of Caf following exposure in rats were evaluated in comparison with oral administration. Furthermore, the applicability of a dried blood spot (DBS)-based approach to pharmacokinetic evaluation in human inhalation studies was explored.

Methods: Caf and its related compounds were analyzed using an Agilent 1260 Infinity II LC system coupled with a 6470 Triple Quad LC/MS. The separation of analytes was performed on a Phenomenex Kinetex Biphenyl column (2.1 × 100 mm, 2.6 µm) using a gradient of 0.1% formic acid in water and methanol. The CSE was prepared according to the CORESTA method. In exposure studies, rat plasma samples were collected over time following exposure to vaporized Caf, and analytical samples were prepared with Caf-d9 as an internal standard. In inhalation studies, DBS samples were collected by finger prick following e-cigarette use. For comparison, oral administration was conducted in both rats and humans. All experiments were conducted with appropriate ethical approvals.

Results: LC-MS/MS analysis revealed that CSE contained Caf along with small amounts of theophylline, theobromine, and paraxanthine. In exposure studies, rapid appearance of Caf in plasma was observed, indicating faster absorption compared with that of oral administration. In inhalation studies, the DBS-based method enabled quantification of Caf from a small volume of blood (ca. 70 µL). Following oral administration, a concentration-time profile of Caf was observed, with 1902.3 ng/mL of C_{max}.

Discussion: These results suggest that the use of e-cigarette products may enable efficient systemic intake of Caf, even with relatively small amounts, and may lead to a more rapid onset of effects compared with oral administration. In exposure studies, rat plasma concentrations of Caf and its metabolites following exposure to e-cigarette smoke were low, likely due to the absence of active smoking behavior, which limited detailed pharmacokinetic evaluation. On the other

hand, the DBS-based method employed in this study is expected to facilitate pharmacokinetic assessment in humans, and further studies using inhalation are currently underway.

Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

P-117

Semi-Synthetic Cannabinoids: The Newest Challenge in Analyzing THC Isomers

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Abstract

Introduction: Over the past several years, Δ8-THC has made headlines as a popular "legal" alternative to Δ9-THC. The rise in popularity of Δ8-THC led to a new analytical challenge for toxicology laboratories—differentiating between the Δ8 and Δ9 isomer forms. For labs performing this testing by LC-MS/MS, complete separation of these isomers and their metabolites is necessary for accurate quantitation. Recently, a new group of compounds referred to as semi-synthetic cannabinoids have emerged on the market. Drug monitoring groups in the U.S. have suggested that these compounds be added to toxicology testing scopes due to increased use and detection. Many toxicology laboratories have already implemented LC-MS/MS methods to simultaneously analyze for Δ8-THC and Δ9-THC in casework. In this work, eight emerging semi-synthetic cannabinoids (9(R)-HHC, 9(S)-HHC, 9(R)-HHC-COOH, 9(S)-HHC-COOH, Δ10-THC, Δ9-THC-O-Acetate, Δ9-THCP, and CBDP) were added to an existing LC-MS/MS method used for the analysis of Δ8-THC, Δ9-THC, and their hydroxy and carboxy metabolites in whole blood.

Objectives: Incorporate eight emerging semi-synthetic cannabinoids into an existing method developed for analysis of THC isomers in whole blood.

Methods: The original method was developed for the quantitative analysis of Δ8-THC, Δ9-THC and their isomeric metabolites in whole blood by LC-MS/MS. The method used a Raptor FluoroPhenyl 100 x 3 mm, 2.7 µm column and mobile phases of water and methanol, both acidified with 0.1% formic acid. The column temperature was 40°C, the flow rate was 0.8 mL/min, and gradient elution was used. A sample containing the eight semi-synthetic cannabinoids and the original six cannabinoids was prepared at a concentration of 100 ng/mL. The sample was run on the original method to determine separation and elution order.

Results: While resolution was achieved for all compounds, including isomers, using the original method conditions, Δ10-THC, Δ9-THCP, and Δ9-THC-O-Acetate eluted at 100% B, where matrix interferences are known to wash off the column. To prevent interference, the method conditions were altered to allow these compounds more time on the column. Additional resolution for the isomers was also achieved by dropping the column temperature from 40°C to 30°C. All critical pairs had a calculated resolution of 1.5 or better, demonstrating baseline resolution was achieved.

Discussion: All eight semi-synthetic cannabinoids were successfully integrated into a pre-existing LC-MS/MS panel for the analysis of THC isomers in whole blood. Because different isomer configurations can have different psychoactive activities, it is important to differentiate between isomer forms in toxicology casework. This work highlights the importance of choosing a stationary phase which shows optimal selectivity for the analyte class being analyzed, allowing new analytes to easily be integrated into the method as they emerge.

Disclosure

Yes, I, or a member of my immediate family, has a financial interest to disclose.

Conflict of Interest

Authors are employed by Restek Corporation

P-118

Simultaneous Analysis of 27 Multi-Class Antidiabetic Drugs in Blood Using LC-MS/MS

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Abstract

Introduction: There are many classes of antidiabetic (Glucose-lowering) drugs, and their chemical structures are increasingly diverse. Recently, inappropriate use of antidiabetic drugs as “weight-loss drugs” has been frequently reported, and this has caused health problems. Therefore, rapid identification of these drugs is important in clinical and forensic settings.

Objectives: This study evaluated the LC–MS/MS method for the analysis of structurally diverse antidiabetic drugs in whole blood.

Methods: Thirty antidiabetic drugs were targeted, including nine DPP-4 inhibitors, six SGLT2 inhibitors, five sulfonylureas, three glinides, three α-glucosidase inhibitors, two biguanides, and a glimin. Calibration standards (0.1–500 ng/mL) were prepared in water using mixed working standard solutions containing the 30 target analytes and were processed using the same QuEChERS procedure as whole-blood samples (non–matrix-matched, procedural calibration) to standardize sample preparation. For accuracy assessment, blank whole blood (100 µL) was fortified with the mixed standard solution (10 µL) to a nominal concentration of 20 ng/mL, and diazepam-d5 (0.2 µg/mL, 20 µL) was added as the internal standard. For QuEChERS extraction, LC/MS grade water (200 µL) and acetonitrile (300 µL) were added with magnesium sulfate (80 mg) and sodium acetate (20 mg), mixed, and centrifuged. The supernatant was evaporated under a nitrogen stream and reconstituted in 100 µL water/methanol (80:20, v/v). LC–MS/MS analysis was performed using a LCMS-8050RX (Shimadzu Corporation).

Results: With the QuEChERS procedure, three highly hydrophilic α-glucosidase inhibitors were hardly recovered. However, good validation results were obtained for the other 27 small-molecule antidiabetic drugs. Good linearity ($R > 0.995$) was obtained over 0.1–100 ng/mL for 3 compounds (omarigliptin, pioglitazone, and Repaglinide), 0.1–200 ng/mL for 9 compounds (anagliptin, alogliptin, saxagliptin, trelagliptin, sitagliptin, acetohexamide, glibenclamide, mitiglinide, and nateglinide), and 0.1–500 ng/mL for the other 15 compounds. In addition, peaks were detected for all compounds even at the low concentration of 0.1 ng/mL. The accuracy for these 27 compounds was within 80–120%. Repeatability, expressed as %RSD, was ≤ 7.9%, indicating that this method provides sufficient precision for the analysis of whole blood.

Discussion: With the QuEChERS procedure, the three highly hydrophilic α-glucosidase inhibitors were not detected at ≤ 200 ng/mL. However, satisfactory results were obtained for the other 27 small-molecule antidiabetic drugs. Acceptable linearity ($R > 0.995$) was obtained 0.1–100 ng/mL for three compounds (omarigliptin, pioglitazone, and repaglinide), 0.1–200 ng/mL for nine compounds (anagliptin, alogliptin, saxagliptin, trelagliptin, sitagliptin, acetohexamide,

glibenclamide, mitiglinide, and nateglinide), and 0.1–500 ng/mL for the other fifteen compounds. The accuracy at the positive control level for these 27 compounds was within 80–120%. Repeatability, expressed as %RSD, was ≤ 7.9% (n=5), indicating that this method provides sufficient precision for the analysis of whole blood.

Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

P-119

Fast Screening for 44 Illicit Drugs in Urine by UHPLC/MS/MS

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Abstract

Introduction: Drugs of abuse refer to the use of illegal and controlled drugs for non-medical purposes, the primary purpose being that these drugs can trigger the user's central nervous system and induce an altered state of mind. Drug abuse has become an important cause of health, economic, and social problems. Therefore, the identification and determination of these drugs in different matrices is essential for forensic and toxicology studies.

Objectives: The main objectives of this work were to develop a rapid LC/MS/MS method for the separation and detection of 44 drug of abuse compounds in urine samples, and to evaluate the selectivity, linearity, and sensitivity of the QSight® 220 LC/MS/MS system. The drugs of abuse included in this study span the classes of narcotics, stimulants, depressants, hallucinogens, cannabis and other drugs of concern.

Methods: Chromatographic separation of analytes from potential interfering components was conducted utilizing a PerkinElmer LX50 UHPLC system, and subsequent determination of analytes was achieved using a PerkinElmer QSight 220 triple quadrupole mass detector with a dual ionization source. All instrument control, data acquisition and data processing were performed using Simplicity™ 3Q software. For sample preparation: 0.5 mL of a urine sample and 2 mL of acetonitrile were added to a 10 mL centrifuge tube and vortex mixed for two minutes. The solution was then centrifuged at 6000 rpm for three minutes. The supernatant was filtered through a 0.22 µm syringe filter prior to LC/MS/MS analysis. Analytes were spiked in urine samples at a concentration of 1.0 µg/L, except for oxazepam and temazepam which were spiked at 2.5 µg/L.

Full list of analytes: 6-acetylmorphine, 7-aminoclonazepam, alprazolam, amitripthyline, amphetamine, benzoylecognine, buprenorphine, clonazepam, cocaethylene, cocaine, codeine, desalkylflurazepam, dextromethorphan, doxepin, EDDP, EMDP, ephedrine, estazolam, ethylmorphine, fentanyl, flunitrazepam, flurazepam, ketamine, lorazepam, MDA, MDEA, MDMA, meperidine, meprobamate, methadone, methamphetamine, midazolam, morphine, nimetazepam, nitrazepam, norketamine, nortriptyline, oxazepam, oxycodone, phencyclidine, temazepam, THC, tramadol, triazolam.

Results: All 44 compounds can be analyzed in a short run time of around 4.5 min. Good linearity was obtained for each analyte ranging from 0.5 to 80 µg/L with correlation coefficient (R2) > 0.997. LOQs ranged from 0.025 µg/L to 2.5 µg/L, depending on the analyte. Recoveries of the analytes from the urine samples ranged from 83.6% to 120.2% with RSD <7%.

Discussion: In this study, an LC/MS/MS method was developed and applied for rapid determination of 44 illicit drugs in urine samples by coupling an LX50 UHPLC with QSight 220 mass spectrometry. The results showed that the method is sensitive and selective with a wide linear range for the studied drugs. Therefore, this method is suitable for fast screening and quantification of these commonly abused drugs in criminal investigations.

Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

P-120

Validated Liquid Chromatography-Tandem Mass Spectrometry Method for Urinary Gonadotrophic-Releasing Hormone (GnRH) Agonists and Metabolites: Monitoring Adherence to Pharmacological Treatment for Libido Suppression

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Abstract

Introduction: Monitoring adherence to pharmacological treatment for libido suppression (chemical castration) is critical for preventing recidivism in sex offenders. However, current analytical methods that rely on testosterone (T)/epitestosterone (E) ratios are limited by physiological variabilities.

Objectives: This study aimed to develop and validate a liquid chromatography-tandem mass spectrometry (LC-MS/MS) method for the direct, simultaneous quantification of three gonadotropin-releasing hormone (GnRH) agonists (leuprolide, goserelin, and triptorelin) and their main metabolites in human urine.

Methods: Urine samples (1000 µL) spiked with an internal standard (10 ng/mL) were centrifuged (45,000 × g, 5 min) and subjected to solid-phase extraction using weak cation exchange (WCX) cartridges. The extracts were evaporated, reconstituted in 50% methanol with formic acid, and analyzed using a UPLC-MS/MS system. Chromatographic separation was achieved on a Waters XSelect HSS T3 column (2.1 × 100 mm, 2.5 µm) using a gradient elution with 0.1% formic acid in water and methanol at a flow rate of 350 µL/min.

Results: All target compounds were separated and analyzed within 10 minutes. The method was validated according to U.S. FDA bioanalytical method validation guidelines. The limits of detection (LOD) and lower limits of quantification (LLOQ) were 0.004–0.13 ng/mL and 0.05–0.5 ng/mL, respectively, with linearity of $r^2 \geq 0.995$. Intra- and inter-day accuracies were within –11.5% to 9.8%, and precisions were ≤ 11.8%. Matrix effects, selectivity, dilution integrity (100-fold), and stabilities (bench-top, autosampler, and long-term) satisfied the acceptance criteria. The applicability of the method was demonstrated through the analysis of forensic urine samples (n = 193).

Discussion: The developed LC-MS/MS method provides adequate sensitivity and selectivity for monitoring GnRH agonist adherence. Direct quantification of these drugs and their metabolites yields definitive evidence of treatment, effectively addressing the limitations of conventional T/E ratio monitoring and supporting the reliable assessment of libido suppression in forensic applications.

Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

Conflict of Interest

Grant/Research Support: This work was supported in part by the Supreme Prosecutors' Office of Republic of Korea.

P-121

Validation of a High-Resolution Mass Spectrometry (LC-HRMS/MS) Method for the Selective Detection and Quantitation of Gamma-Hydroxybutyrate (GHB) in Antemortem Urine

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Abstract

Introduction: Gamma-hydroxybutyrate (GHB) is a potent central nervous system depressant often implicated in drug-facilitated crime (DFC). Forensic interpretation is challenged by GHB's endogenous presence (typically <10ug/ml in urine) and its rapid elimination window which necessitates a sensitive methods with clear cut-off values.

Objectives: This poster presents highlights from the development and full validation of an LC- HRMS/MS-based method designed to distinguish exogenous intake from endogenous levels in antemortem urine samples.

Methods: A matrix-matched calibration curve, in-house quality control samples, and an independent external quality control were prepared using a simple liquid-liquid extraction (LLE) with ethyl acetate, utilising GHB-d6 as an internal standard. Analytical separation was achieved via ultra-high performance liquid chromatography (UHPLC) using a 5-minute isocratic elution method. A C18 BEH column with 1.7um particles allowed for the resolution of GHB from its precursors and structural isomers while achieving a short analysis time. Detection was performed on a Q Exactive Orbitrap (Thermo Fisher Scientific) operating in parallel reaction monitoring (PRM) mode. Method validation was conducted in accordance with SWGTOX (2013) and ANSI/ASB Standard 036 (2019) guidelines, assessing selectivity, linearity, accuracy, precision, and matrix effects.

Results: The method demonstrated excellent linearity (i.e. 4.13 – 132ugs/ml) with a correlation coefficient (R^2) of > 0.98. The lower limit of quantitation (LLOQ) was established at 4.13ug/ml which is significantly below the standard 10ug/ml forensic cut-off. Intra-day and inter-day precision and accuracy were within ±15% (and ±20% at LLOQ). No significant matrix interference from common drugs or endogenous isomers was observed. Stability studies confirmed processed sample integrity of more than 72 hrs under refrigerated (4^0C) conditions. The measurement of uncertainty was calculated to be ±15%.

Discussion: This validated HRMS/MS method provides a robust, sensitive and specific tool for GHB detection and quantitation in antemortem urine. The high mass accuracy of the LC-HRMS/MS minimizes false positives from endogenous isomers, while the LLOQ supports extended detection windows in forensic investigations of suspected GHB exposure.

Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

P-122

Identification of a Unique Thermal Artifact at m/z 134.09477 as a Forensic Marker for Catha Edulis Using DART-ToF-MS

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Abstract

Introduction: Although *Catha edulis* (Khat) has been extensively studied, the chemical instability of its psychoactive alkaloids remains a bottleneck for forensic screening. This research investigates ambient ionization mass spectrometry as a rapid alternative, specifically focusing on identifying diagnostic artifacts that enhance the reliability of real-time forensic identification.

Objectives: The primary objectives of this study were to utilize DART-ToF-MS for the rapid screening of Khat constituents without prior extraction, characterize a unique diagnostic thermal artifact (m/z 134.09477) as a forensic marker, and establish a spatial distribution profile of alkaloids to optimize forensic sampling protocols.

Methods: Fresh samples were analyzed directly via a DART ion source (350°C helium stream) coupled with high-resolution ToF-MS. Leaves and stems were introduced without pretreatment. Molecular formulas were assigned via accurate mass measurements (error < 2 mDa) and confirmed through isotopic pattern verification.

Results: Beyond the detection of cathinone and cathine, high-resolution analysis enabled the precise characterization of a prominent peak at m/z 134.09477 ($C_9H_{12}N$), identified as a diagnostic artifact. Spatial mapping revealed that the upper stem maintains a higher alkaloid concentration than leaves, providing a critical target for targeted forensic sampling.

Discussion: The identification of the m/z 134 artifact, characterized as a diagnostic dehydration product derived from cathine, provides a novel “chemical signature” that significantly elevates identification reliability. This ion results from the in-source loss of a water molecule during the ionization process. DART-ToF-MS offers lab-grade results in real-time, making it an ideal tool for high-throughput screening. Integrating these artifacts into official forensic protocols can reduce false interpretations in seized materials at border control points.

Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose

P-123

Use of a New Derivatizing Method to Quantify Phosphatidylethanol by Gas Chromatography-Electron Impact-Mass Spectrometry

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Abstract

Introduction: Phosphatidylethanol (PEth) is a phase II biomarker formed in the walls of red blood cells that shows excellent sensitivity and specificity as a screening biomarker for chronic alcohol use and abuse. This new derivatization method differs from our previous version by increasing the size of the derivatizing group from trimethylsilyl to t-butyl-dimethylsilyl. This work is based on US patent 11,085,939 B2 titled “Quantifying Phosphatidylethanol from Blood Samples” published in 2021.

Objectives: The goal of this work was to develop a “point of care” means of detection and quantitation of PEth to identify alcohol misuse by patients in a clinical setting.

Methods: PEth was derivatized with N-tert-butyldimethylsilyl-N-methyltrifluoroacetamide (MTBSTFA) to produce the corresponding disubstituted ethylphosphate analyte. Chloroform solutions of PEth 16:0/18:1 (numerical designations used to indicate fatty acid residue structures) and phosphatidylethanol-d5 16:0/18:1 (PEth-d5) were derivatized with MTBSTFA + 1% TBDMCS. Samples were analyzed by GC-EI-MS on an Agilent 7890A/5975C and 8890/5977 in full scan/SIM dual mode. Quantitative analysis was performed to establish linearity in the range of 10-9000 ng/mL.

Results: The derivatization of PEth using MTBSTFA produced an analyte that generated a new mode of fragmentation compared to MSTFA. In addition to the characteristic M-15 loss of methyl fragment observed with TMS-derivatization, the new technique generated a strong (base ion) M-57 loss of t-butyl fragment. The strength of the new fragmentation signal resulted in greater analyte specificity and allowed for quantification of PEth in a clinically relevant range. Linear quantitative analysis was established in the range of 10-9000 ng/mL.

Discussion: This work has established that the new form of derivatization imparts greater specificity to the analyte and provides linear quantitation of PEth down to 10 ng/mL. The technique has proven to be effective at measuring the total amount of ethanol incorporated into the blood phospholipids in whole, dried, and potentially decomposed blood (e.g. postmortem).

Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

P-124

Chronological Prevalence of Δ^9-THC-COOH and Δ^9-THCV-COOH from 1999-2020

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Abstract

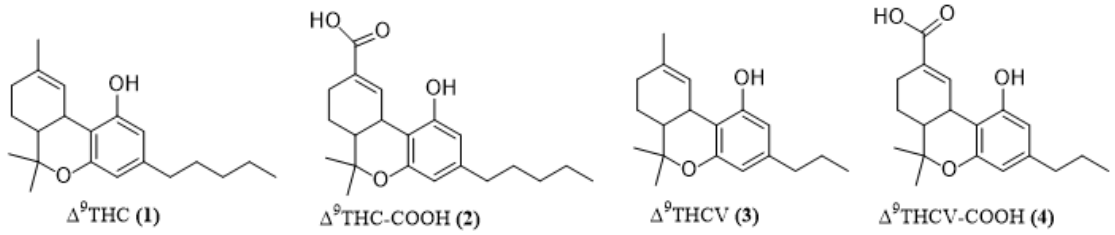
Introduction: The legalization of dronabinol (synthetic Δ^9-THC (1), marketed as Marinol®) and other synthetic or purified forms of Δ^9-THC (1), together with the recent surge in hemp products, has made it difficult to determine whether the Δ^9-THC-COOH (2) metabolite of Δ^9-THC (1) in a urine sample reflects ingestion of either synthetic Δ^9-THC (1) or of cannabis plant material. Simply detecting Δ^9-THC-COOH (2) on its own cannot distinguish between Marinol® or cannabis plant material use, so a cannabinoid marker was needed to better determine which one of the two sources the Δ^9-THC (1) present in the sample came from.

Objectives: Δ^9-tetrahydrocannabivarin (Δ^9-THCV, 3) is present in cannabis plant material but not in dronabinol. Therefore, its metabolite, Δ^9-tetrahydrocannabivarin acid (Δ^9-THCV-COOH, 4), was chosen to be able to distinguish the two sources: the presence of both Δ^9-THCV-COOH (4) and Δ^9-THC-COOH (2) indicates the use of cannabis plant material, whereas Δ^9-THC-COOH (2) without Δ^9-THCV-COOH (4) points to synthetic Δ^9-THC (1) use. This study reports the chronological prevalence of Δ^9-THC-COOH (2) and Δ^9-THCV-COOH (4) in urine from 1999–2020 across all 50 states.

Methods: Data from 1,643 urine samples analyzed by GC/MS at ElSohly Laboratories and collected from 1999–2020 were reviewed for trends in the concentrations of Δ^9-THC-COOH (2) and Δ^9-THCV-COOH (4). The data was organized through four different ways: by state; by chronological trends in the concentrations of both metabolites; by the proportion of samples positive for Δ^9-THCV-COOH (4); and by sorting samples into four concentration tiers for Δ^9-THCV-COOH (4) over 1999–2020 (≤25 ng/mL, 26–50 ng/mL, 51–100 ng/mL, and >100 ng/mL).

Results: By state, concentrations of both metabolites were generally under 500 ng/mL. Fourteen states had samples averaging 500–1000 ng/mL for Δ^9-THC-COOH (2), one state (ND) averaged about 1750 ng/mL, and two states (DE and NM) averaged 2000–2500 ng/mL. Over time, the relationship between the two metabolites remained approximately the same, with increases or decreases in Δ^9-THC-COOH (2) proportional to those in Δ^9-THCV-COOH (4). The proportion of samples positive for both metabolites generally stayed above 60%; however, the rate fell and rose again every few years. Across the four concentration tiers, few to no samples were positive for Δ^9-THCV-COOH (4) below 25 ng/mL; samples were positive about 40–70% in the 26–50 ng/mL tier; 50–80% positive in 2002, 2005, and 2006 in the 51–100 ng/mL tier; and 60–95% positive in almost every year above 100 ng/mL.

Discussion: Through the organization of all 1999–2020 data by these four methods revealed consistent trends in the concentrations of Δ^9-THC-COOH (2) and Δ^9-THCV-COOH (4), with the concentrations of both metabolites rising and falling together throughout. Because the samples were taken from states all across the country, the dataset gives an excellent and appropriate representation of how these concentrations vary with geography, as well as with the cannabis varieties and strains found across the U.S., D.C., and Puerto Rico.



Δ^9THC **(1)** Δ^9THC-COOH **(2)** Δ^9THCV **(3)** Δ^9THCV-COOH **(4)**

Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

P-125

Retrospective Review of Mitragynine-Positive Casework in Dallas County for Mitragynine Pseudoindoxyl

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Abstract

Introduction: The use of kratom, the herbal substance derived from the leaves of the *Mitragyna speciosa* tree, has increased in prevalence, which has resulted in the need for specialized testing for the main psychoactive alkaloids associated with this plant. Mitragynine is the primary psychoactive component of kratom, commonly sought after for its stimulant and opioid-like effects on the body. Its metabolite, 7-hydroxymitragynine, is another alkaloid of interest. Currently, the Toxicology Laboratory at the Dallas County Southwestern Institute of Forensic Sciences (SWIFS) quantitates mitragynine by LC-MS/MS and reports 7-hydroxymitragynine qualitatively from a LC-QTOF-MS/MS drug screen.

Mitragynine pseudoindoxyl (MP), another metabolite of mitragynine, has recently emerged as a new biomarker of interest to indicate kratom use due to the known instability of 7-hydroxymitragynine. MP is also an emerging component in drug products. Both 7-hydroxymitragynine and MP are isolated or synthesized from mitragynine and marketed as "kratom-based" products due to their higher potency compared to mitragynine. The Toxicology Laboratory at SWIFS added MP to the scope of the LC-QTOF-MS/MS drug screen for qualitative reporting in late 2025.

Objectives: To perform a retrospective analysis for MP in 2025 mitragynine positive postmortem casework and compare the performance against 7-hydroxymitragynine in the same casework.

Methods: For this introductory study, postmortem cases positive for mitragynine in blood by the quantitative LC-MS/MS method between January 1, 2025, and December 31, 2025, were retrospectively analyzed for MP in the drug screen by LC-QTOF-MS/MS.

The LC-QTOF-MS/MS targeted screening method is used for the screening of mitragynine and its metabolites. If positive, mitragynine is confirmed by the quantitative LC-MS/MS method and the metabolites (7-hydroxymitragynine and MP) are confirmed on the QTOF drug screen following positive identification in a second sample, preferably on a second blood tube. Reporting criteria for the qualitative identification of 7-hydroxymitragynine and MP includes retention time, peak asymmetry, signal to noise ratios, mass error, and the case sample mass spectra is supported by a library match from the in-house library.

Results: Retrospective data analysis was performed on 32 samples. 7-hydroxymitragynine was reported in 50% (n=16) of mitragynine positive cases. MP was identified in 88% (n=28) of the same casework. In 12 cases, MP produced more gaussian shaped chromatography that was easily identifiable compared to the 7-hydromitragynine peak. In addition, the MP in these cases produced mass spectrum that matched the Laboratory's in-house library when the 7-hydroxymitragynine mass spectrum either was not produced or produced a poor spectrum that did not meet acceptance criteria. Overall, MP identification indicates it as a better performing biomarker than 7-hydroxymitragynine in the Laboratory's LC-QTOF-MS/MS method.

Discussion: Introducing MP into the LC-QTOF-MS/MS drug screen has enhanced the Toxicology Laboratory's abilities to detect mitragynine use in postmortem casework when the parent mitragynine is not identified during screening. MP's increased performance in the Laboratory's method compared to 7-hydroxymitragynine indicates MP may be more suitable biomarker for other laboratories monitoring for kratom use.

Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

P-126

Lactate-Dependent Tau K243 Lactylation Deficiency Impairs Hippocampal Mitochondrial Function Mediating Ketamine-Induced Neurotoxicity and Cognitive Impairment

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Abstract

Introduction: Chronic ketamine abuse can induce severe hippocampus-dependent neurotoxicity and cognitive dysfunction, yet the underlying molecular mechanisms remain incompletely elucidated. Lactate-driven lysine lactylation is a recently identified post-translational modification that couples cellular metabolic homeostasis with neuronal functional regulation, but its role in ketamine-related neurotoxicity remains completely unknown.

Objectives: This study aims to systematically dissect the metabolic–post-translational modification regulatory axis underlying ketamine-induced cognitive impairment and identify novel therapeutic targets.

Methods: Using a mouse model of chronic ketamine exposure and HT22 hippocampal neuronal cells, we integrated untargeted metabolomics, lactylation proteomics, molecular biology, in vivo AAV-mediated targeted intervention, and behavioral analyses to conduct systematic mechanistic studies.

Results: Chronic ketamine exposure significantly downregulated the expression of the neuronal monocarboxylate transporter MCT2, disrupted the astrocyte–neuron lactate shuttle system, and led to a marked decrease in neuronal lactate levels. Lactate deficiency specifically reduced lactylation of Tau protein at lysine 243 (K243), without affecting total Tau protein expression. Decreased Tau K243 lactylation directly induced mitochondrial structural disorganization, membrane potential depolarization, reduced ATP production, and ROS accumulation, ultimately mediating hippocampal neuronal injury and cognitive dysfunction. Exogenous sodium lactate supplementation significantly reversed the above pathological processes induced by ketamine, and this neuroprotective effect was completely dependent on Tau K243 lactylation.

Conclusion: This study reveals for the first time that downregulation of Tau K243 lactylation is a key molecular event in ketamine-induced neurotoxicity and cognitive impairment, providing a novel therapeutic target and strategy for the prevention and treatment of ketamine abuse-related neurotoxicity.

Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

Conflict of Interest

National Natural Science Foundation of China (82502278, 82030057, 82072111)

P-127

Presence of Novel Psychoactive Substances in Negative Confirmed Benzodiazepine Clinical Urine Specimens

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Abstract

Introduction: Designed to detect distinctive molecules for specific drug classes, immunoassay is a reliable technique that is routinely used for benzodiazepine detection in urine. However, novel psychoactive substances (NPS) challenge the dependability of this standard method by generating presumptive positive results for traditional benzodiazepines. Due to their structural similarities, immunoassays are unable to differentiate between traditional and novel benzodiazepines. Furthermore, immunoassay panels have remained stagnant, which makes the prevalence of NPS in urine drug screenings unknown.

Objectives: The objective of this surveillance study was to determine the prevalence of NPS in clinical urine specimens that screened positive and confirmed negative for traditional benzodiazepines.

Methods: Clinical urine specimens from across the United States were submitted for traditional immunoassay drug screening using an EMIT II Benzodiazepine immunoassay kit. Specimens that screened as presumptive positive were reflexed to an LC-MS/MS confirmatory method for traditional benzodiazepines. Specimens that screened positive but confirmed negative for traditional benzodiazepines were targeted for this study. These specimens were deidentified and analyzed for NPS using an LC-MS/MS method.

Semi-quantitative analysis was used to identify 100 NPS in the following 6 classes: fentanyl analogs (e.g. carfentanil), designer benzodiazepines (e.g. bromazolam and desalkylgidazepam), designer opioids (e.g. nitazenes and orphines), designer stimulants (e.g. pentylone and isopropyl butylone), synthetic cannabinoids (e.g. the MDMB-4en-PINACA metabolite), and other substances (e.g. xylazine, medetomidine, and tianeptine). Cutoff concentrations ranged from 1 ng/mL to 20 ng/mL for all NPS.

Results: In total, 1,424 specimens that screened positive and confirmed negative for traditional benzodiazepines were evaluated for the presence of NPS. Of these, 170 (11.9%) tested positive for at least 1 NPS. Of the NPS-positive specimens, 87 (51.2%) were positive for 2 or more NPS classes. The predominate NPS classes detected included designer benzodiazepines (134 specimens or 78.8% of positives) and other substances (93 specimens or 54.7% of positives). Of those specimens positive for a designer benzodiazepine, hydroxy-bromazolam was detected in 104 (77.6%) specimens and bromazolam was detected in 95 (70.9%) specimens. Of the specimens positive for other substances, xylazine was detected in 76 (81.7%) specimens and hydroxy-medetomidine was detected in 42 (45.2%) specimens.

Discussion: This study demonstrates the importance of including NPS in drug monitoring testing to identify analytes that can go undetected with traditional panels. With the constant emergence of new and different NPS, there is a necessity to have detection methods that can identify these ever-evolving drugs. Patient compliance with drug monitoring programs and

creating an effective recovery treatment plan relies on receiving the most accurate test results. This can only be achieved when comprehensive tests are available to detect substances that may be unknown even to the patient.

Disclosure

Yes, I, or a member of my immediate family, has a financial interest to disclose.

Conflict of Interest

Salary

P-128

Cross-Reactivity of Novel Psychoactive Substances Using Immunoassay and Drug Screen Urine Collection Devices

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Abstract

Introduction: Novel psychoactive substances (NPS) include substances designed to evade detection and drug laws due to their constantly changing nature. NPS are becoming increasingly popular due to their increased potency and psychoactive effects. Immunoassay and urine drug screening cups are screening mechanisms used as a rapid way to obtain a presumptive result for a drug class (e.g. benzodiazepines, opiates, cannabinoids). These screening methodologies do not give information about specific analyte positivity and their cross-reactivity with NPS is relatively unknown.

Objectives: To determine NPS cross-reactivity within evaluated immunoassay drug screens and a drug screen urine collection device.

Methods: Ninety different NPS compounds from 3 NPS categories (designer benzodiazepines [n=29], fentanyl analogs [n=29], designer opioids [n=32]), were spiked into certified drug free urine at 3 concentrations (1000 ng/mL, 100 ng/mL, 10 ng/mL) and analyzed across a 7-panel immunoassay drug screen. The 7-panel screen consisted of Siemens EMIT® II Plus for benzodiazepines, opiates, and methadone (100 ng/mL cutoffs), Thermo CEDIA® buprenorphine (5 ng/mL), Thermo DRI® oxycodone (100 ng/mL) and cannabinoids (20 ng/mL), and ARK™ fentanyl II (1 ng/mL).

NPS cross-reactivity using the Abbott iScreen™ Urine Test DX Drug Screen Tox Cup (amphetamine, barbiturates, buprenorphine, benzodiazepines, cocaine, fentanyl, MDMA, methadone, opiates, methamphetamine, oxycodone, PCP, tricyclic antidepressants, and THC) was also assessed using 54 NPS compounds from the designer benzodiazepine (n=25) and fentanyl analog (n=29) classes. Individual fortified concentrations at 1000 ng/mL were placed in the cup. If the screen was positive (i.e. cross-reactive), lower concentrations (500 ng/mL and 100 ng/mL) were challenged.

Results: On the 7-panel immunoassay drug screen, 22 (76%) designer benzodiazepines screened positive for benzodiazepines at a concentration of 100 ng/mL; 4 screened positive at 1000 ng/mL only; 3 were negative at all concentrations. A total of 18 (62%) fentanyl analogs screened positive for fentanyl at all concentrations. Three fentanyl analogs screened negative. In the designer opioid category, all screened negative at all concentrations. However, at 1000 ng/mL, 3 designer opioids (dipyanone, isotonitazene, and metonitazene) screened positive for buprenorphine and 4 designer opioids were borderline negative (2-methyl-AP-237, brorphine, protonitazene, and pyrrolidino etonitazene).

When using the drug screen urine cups, 16 of the 25 designer benozdiazepines (64%) were positive for benzodiazepines at 1000 ng/mL, 14 (56%) at 500 ng/mL, and 2 (8%) at 100 ng/mL. Many of the designer benzodiazepines showed possible cross-reactivity with amphetamine. Of the 29 fentanyl analogs tested in the iScreen™ cups, 20 (69%) were positive for fentanyl at 1000 ng/mL, 19 (66%) at 200 ng/mL, and 8 (28%) at 10 ng/mL.

Discussion: Although some NPS classes have significant cross-reactivity in current screening immunoassays and/or urine collection devices, major gaps in illicit drug detection remain. In this study none of the designer opioid compounds screened positive at any concentration. This study also showed other gaps in NPS detection using screening methodologies, including lack of cross-reactivity with certain designer benzodiazepines, like clonazolam-stuctured analytes, and fentanyl analog metabolites, like norcarfentanil. These results may provide insight into the illicit drugs that physicians could be missing by utilizing screen-only methodologies for patient testing.

Disclosure

Yes, I, or a member of my immediate family, has a financial interest to disclose.

Conflict of Interest

The authors are paid salary and may own stock for Quest Diagnostics.

P-129

Synthesis of 25E-NBOH Metabolites and Their Identification in Biotransformation Models

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Abstract

Introduction: 25E-NBOH is a novel psychoactive substance with potent psychedelic effects. Although its metabolism has been previously investigated, reported metabolites have not been confirmed using authentic analytical standards. In addition, inconsistencies exist across the literature regarding the main intoxication biomarkers, and stereochemical aspects of metabolites remain largely unaddressed.

Objectives: The aim of this study was to perform a comprehensive, standard-supported investigation of 25E-NBOH metabolism, to confirm previously reported metabolites, identify reliable intoxication biomarkers, and compare findings with existing literature. A further objective was to develop synthetic strategies for reference standards, including deuterated analogues.

Methods: Biotransformation of 25E-NBOH was investigated using Wistar rats, pooled human liver microsomes supplemented with an NADPH-regenerating system, and cultures of *Cunninghamella elegans* as an environmentally and ethically favorable alternative model. Samples were analyzed using UHPLC coupled to high-resolution tandem mass spectrometry (Orbitrap HRMS) operating in positive electrospray ionization mode with data-dependent MS/MS acquisition. Metabolite annotation was performed using Compound Discoverer software based on accurate mass measurements, predicted biotransformations and MS/MS fragmentation data. Selected metabolites were unequivocally identified by comparison with in-house synthesized analytical standards. Novel synthetic pathways were developed for previously unreported metabolites, as well as for deuterated NBOH derivatives starting from phenol-d_6.

Results: A total of 56 metabolites were annotated, of which eight were unequivocally identified using authentic analytical standards synthesized for the first time. The main intoxication biomarkers were identified and systematically compared with previously published data, resolving several inconsistencies in reported metabolic profiles. The proposed synthetic approaches enabled efficient preparation of both non-labeled and deuterated reference standards. Although pharmacokinetic parameters were not assessed, the recurrence and abundance of these metabolites across models supported their suitability as analytical biomarkers.

Discussion: This study provides a comprehensive and experimentally validated metabolic profile of 25E-NBOH. The identification of key biomarkers supported by analytical standards improves confidence in toxicological screening. The comparison with existing literature highlights discrepancies in previously reported data and emphasizes the importance of standard-based confirmation. Additionally, the developed synthetic methodologies offer a versatile platform for the preparation of reference standards applicable to a broader range of

phenethylamine derivatives.

References

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Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

P-130

Metabolite Identification of a Difluorinated MDMA Derivative (DFMDMA) Using Human Liver Microsomes via UHPLC-HRMS/MS

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Abstract

Introduction: 3,4-methylenedioxymethamphetamine (MDMA) is a substance commonly associated with recreational use as a party drug. However, in recent years, it has also been the subject of intensive research regarding its potential therapeutic use as an adjunct in psychotherapy, particularly in the treatment of post-traumatic stress disorder. MDMA is metabolized in the liver by cytochrome P450 enzymes into highly biochemically reactive catechol metabolites which may undergo autooxidation or be further metabolized into o-quinones, which are compounds capable of generating reactive oxygen species. Fluorinated MDMA derivatives are expected to follow different metabolic pathways compared to the parent compound, particularly in Phase I oxidative reactions, which may result in the formation of different or as yet unknown metabolites.

Objectives: This study aimed to characterize the metabolism of DFMDMA through in vitro biotransformation experiments using human liver microsomes (HLM). The biological samples were analyzed using Ultra-High Performance Liquid Chromatography coupled with High-Resolution Tandem Mass Spectrometry (UHPLC-HRMS/MS). The metabolic profile of DFMDMA was compared with that of MDMA, thereby confirming or refuting the hypothesis that the fluorinated structural modification leads to the formation of different metabolites.

Methods: The HLM samples were prepared in triplicate at three time points by terminating the biotransformation reaction with the addition of cooled acetonitrile at 20, 40, and 60 minutes. All samples were subsequently prepared by removing the supernatant, evaporating the residue, and reconstituting the residue in the initial mobile phase. The samples were then analyzed using a Thermo Scientific™ Vanquish™ liquid chromatograph coupled with an Orbitrap Exploris 120 mass spectrometer equipped with an Heated Electrospray Ionization (H-ESI+) ion source operating in positive ion mode (Thermo Fisher Scientific, USA). Metabolites were proposed using Compound Discoverer software (Thermo Fisher Scientific, USA), and structures were confirmed by comparing the fragmentation spectra and retention times of synthesized (reference) metabolites with those of the proposed metabolites.

Results: Preliminary UHPLC-HRMS/MS analysis confirmed the biotransformation of DFMDMA within the HLM incubation system. Based on accurate mass measurements and characteristic MS/MS fragmentation patterns, several Phase I metabolites were identified. The primary metabolic pathways observed include N-demethylation to diF-methylenedioxyamphetamine (DFDA) and hydroxylation of the aromatic ring or the side chain. The influence of the fluorine substituents on metabolic stability and fragment distribution is currently undergoing detailed structural elucidation. A comprehensive metabolic map will be presented.

Discussion: The identified metabolic profiles suggest that fluorine substitution significantly influences the enzymatic affinity and metabolic stability of DFMDMA. These structural modifications may lead to distinct pharmacokinetics and potentially modify the toxicological profile of the compound. The output of this work is a metabolic map of DFMDMA and a set of experimental data that can serve as a basis for further pharmacological studies, as well as for the forensic identification of this substance in biological samples.

Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

P-131

Use of Retrospective TOF Data-Mining to Assess Changes in Kratom Alkaloids in Authentic Toxicology Casework

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Abstract

Introduction: Use of Kratom derived products have increased steadily, particularly over the last decade, in the United States (US). Although traditional ethnobotanical applications include chewing on the leaves of *Mitragyna speciosa* or brewing them into a tea, there is a thriving commercial market that includes concentrated extracts, pressed tablets, and more. With use of kratom, mitragynine is the primary alkaloid with concentration dependent effects, while 7-hydroxy mitragynine (7-OH) and mitragynine pseudoindoxyl (MP) are known metabolites. 7-OH is also a minor alkaloid of the kratom plant and is very unstable, enzymatically transforming to MP. Kratom companies are now tinkering with formulas, producing semi-synthetically enhanced products with 7-OH or MP as the primary constituent due to their greater opioid receptor activity compared to mitragynine, along with completely synthetic kratom compounds such as dihydro-7-hydroxy mitragynine (MGM-15).

Objectives: This research uses retrospective mining of liquid chromatography time-of-flight mass spectrometry (LC-TOF/MS) screening data to identify the changes of kratom alkaloid distribution over time in response to the increased availability of synthetically enhanced kratom products.

Methods: Data obtained from authentic casework submitted to NMS Labs for toxicology testing between December 2024 and December 2025 and analyzed by liquid chromatography time of flight mass spectrometry (LC-TOF/MS) were retrospectively reprocessed for mitragynine, 7-OH, MP, and MGM-15 to review relative responses and combinations of the alkaloids. Data from one screening instrument was used as representation of the larger dataset. An abbreviated dataset from a quantitative confirmation assay for the same four compounds was used as a complement to the screening data.

Results: Data from a total of 528 analyses of blood, serum/plasma, and urine samples that requested comprehensive LC-TOF/MS screening were evaluated as a representative population. Of the 17 kratom-related identifications in December 2024, 94% of cases had mitragynine as the dominant alkaloid; only one case involved MP as the primary constituent in biological matrix. In 2025, there were 512 kratom-related identifications with mitragynine as the major alkaloid in approximately 70% of cases (n=358). 7-OH was the dominant alkaloid in 49 cases (9.5%) while MP was the dominant alkaloid in 96 cases (18%). MGM-15 was the dominant kratom-related alkaloid in 9 cases. In December 2024, only 17% of kratom-related cases reported mitragynine below the screening threshold in the presence of other kratom alkaloids. That percentage increased to 25% for 2025.

In 65 blood samples that reported one or more of these compounds through a confirmation panel, there were 7 cases where mitragynine was not reported but 7-OH, MP, and/or MGM-15 were reported. MP was reported at an average blood concentration of 87 ng/mL (9.6-180 ng/mL); 7-OH was reported in 5 of these same cases, and MGM-15 in two.

Discussion: The toxicology data indicates the growing usage of adulterated kratom products, showing cases with significant amounts of 7-OH and MP in comparison to mitragynine, as well as an uptick in the presence of MGM-15. Synthetic and semi-synthetically adulterated kratom products appear to be involved in an increasing number of adverse events, which is supported by this data collected in medicolegal investigations.

Disclosure

Yes, I, or a member of my immediate family, has a financial interest to disclose.

Conflict of Interest

Salaried employee of NMS Labs

P-132

Evaluation of Three SelectraCore® HPLC Columns for the Separation of Orphine Analogues

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Abstract

Introduction: Orphine analogues, or benzimidazole-2-ones, are potent synthetic opioids that have increased in prevalence following the stricter control of nitazenes in China. These compounds are Novel Psychoactive Substances (NPS) appearing in seized drug and toxicology samples with increasing frequency over the last few years. Due to their high potency, their overdose potential is a cause for worry. Little literature currently exists on orphines but incorporating them into routine toxicological analysis can provide useful insights into their use and trends. The development of a comprehensive analytical method is therefore required to enable their inclusion in routine casework.

Objectives: Assess the separation and behavior of orphine and 11 of its analogues on SelectraCore® columns of three different chemistries with multiple mobile phase combinations.

Methods: Analysis was performed on a Shimadzu Nexera LC-30AD with MS-8050. Three column chemistries were assessed: SelectraCore® C18, DA (biphenyl), and PFPP. The dimensions of each column were 100 x 2.1 mm with a particle size of 2.7 µm. A 30-minute binary gradient was used to determine separation on each column. Mobile phase combinations explored included 0.1% formic acid in water paired with 0.1% formic acid in acetonitrile or methanol and 5 mM ammonium formate and 0.1% formic acid in water paired with 0.1% formic acid in acetonitrile or 5 mM ammonium formate and 0.1% formic acid in methanol. This equates to four different mobile phase combinations. Neat standards were prepared in 10% methanol or 10% acetonitrile containing 25 ng/mL of 5,6-Dichloro brorphine (SR-14968), 5,6-Dichloro desmethylchlorphine (SR-17018), 5,6-dichloro nor-orphine, Brorphine, Chlorphine, Cychlorphine (N-propionitrile chlorphine), Fluorphine, Iodorphine, Nor-orphine (n-desalkyl orphine), Orphine, Spirobrorphine, and Spirochlorphine (R-6890). The source parameters, MRM transitions, gradient, flow rate, column oven temperature, and injection volume were maintained throughout data collection.

Results: Chromatography from analysis with each column and mobile phase combination will be shared. Analytes were successfully eluted using all columns and mobile phase combinations. Column interaction and elution order varied between columns and mobile phases. Including ammonium formate as an additive in the mobile phase improved analyte signal and greatly improved chromatography on SelectraCore® PFPP. Regardless of column chemistry, using methanol as the organic mobile phase improved sensitivity and displayed better separation, while acetonitrile resulted in earlier elution times.

Discussion: SelectraCore® columns are superficially porous allowing for efficient analysis without high back-pressures. The SelectraCore® column is offered in three chemistries: C18, DA (biphenyl), and PFPP, all of which can be used to analyze orphines. Any of these columns and the mobile phase combinations tested can be implemented to analyze orphines using a mass selective detector. Using the scouting gradient tested as a starting point, shorter and more efficient acquisition methods can be developed for use in routine casework.

Disclosure

Yes, I, or a member of my immediate family, has a financial interest to disclose.

Conflict of Interest

Salary

P-133

Strategic Prevention and Reduction of Drug Misuse Using Knowledge Graphs (SPAR-KG): A Knowledge Graph Dashboard for Detecting Emerging Drug Trends in Social Media

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Abstract

Introduction: The rapid growth of drug-related content across social media platforms has created an urgent need for tools that can support the timely detection, monitoring, and interpretation of emerging substance-use trends. In response to this challenge, we present the SPAR-KG Dashboard, an interactive knowledge graph–driven platform designed to analyze drug-related discourse and behavioral signals across Reddit, TikTok, and YouTube. The dashboard integrates multi-platform social media data with semantic analysis, behavioral modeling, and visual analytics to support exploratory research and public health surveillance.

Objectives: This presentation will demonstrate the architecture, analytic capabilities, and use cases of the SPAR-KG Dashboard, highlighting how knowledge graph technologies and ontology-guided analysis can enhance the detection and interpretation of emerging drug trends in online environments.

Methods: A key feature of the dashboard is the incorporation of ontology-based behavioral annotation. Using a Behavior Pattern Ontology developed through the KNowledge Acquisition and Representation Methodology (KNARM), the platform labels behavioral settings, emotional cues, reinforcement patterns, and contextual drivers reflected in video content. These semantic annotations enable more structured querying and interpretation of substance-related behaviors beyond keyword-based analysis alone.

Results: The platform illustrates how semantically enriched dashboards can support researchers, analysts, laboratories, and public health stakeholders in understanding the evolving behavioral landscape of drug-related social media content.

Discussion: The SPAR-KG Dashboard brings together multiple analytic pipelines within a unified interface. Reddit data from drug-focused communities are processed using lexical detection, semantic expansion, and embedding-based similarity analysis to identify related and evolving drug terminology, while temporal visualizations reveal short- and long-term shifts in drug mentions. For TikTok and YouTube, the system integrates captions, comments, audio transcripts, and on-screen text to support multimodal analysis of drug-related content. Sentiment analysis, topic analysis, and risk scoring are used to identify high-risk, high-engagement, and potentially influential videos. Within laboratory settings, the dashboard can function as an early-warning and decision-support tool by highlighting emerging substances, evolving slang, and behavioral signals that may inform targeted screening, method development, retrospective review, and coordination with public health and forensic partners. The dashboard can be integrated into laboratory workflows as a hypothesis-generating surveillance layer rather than a replacement for

confirmatory testing. Important limitations remain: social media data are noisy and not population-representative, platform access and terminology evolve rapidly, and automated multimodal models may still produce false positives or miss coded references. Even with these limitations, the system can improve situational awareness and enable earlier public health messaging, harm-reduction interventions, and more responsive laboratory surveillance, thereby supporting efforts to reduce drug misuse.

Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

P-134

Drug Trends in North Louisiana: A Five-Year Review

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Abstract

Introduction: The North Louisiana Crime Laboratory system supports agencies across 29 parishes, encompassing the northern half of the state. Toxicology casework includes driving under the influence/drugs (DUI/DUID), death investigations, and drug-facilitated crimes. A retrospective analysis was performed to determine the most prevalent drugs and the emergence of novel psychoactive substances (NPS).

Objectives: The objective of this study was to evaluate drug trends in North Louisiana over a five-year period (2021–2025).

Methods: A total of 4,532 cases were completed and reported during the study period. Comprehensive testing was performed on all cases, with no stop-testing protocols applied. Each case underwent alcohol and drug screening followed by confirmatory and/or quantitative analysis as appropriate.

Targeted drug screening and confirmation/quantitation was conducted using a Waters Xevo G2-XS QTOF and a Waters Xevo TQD. The scope of analysis included approximately 125 analytes. Variables included detected drugs, concentrations (when applicable), and specimen type.

Results: The most frequently detected drugs remained consistent throughout the study period. Overall percentages for the 5-year span are included: THC and metabolites (21.9%), methamphetamine/amphetamine (11.3%), fentanyl/Norfentanyl (3.8%), cocaine and metabolite (3.2%), gabapentin (2.4%), and alprazolam (2.3%). A result of no drugs detected represented 11.9% of reported results.

In contrast, NPS trends demonstrated more notable variability; however, they represent a much lower population of total reported results, representing only 3% of reported results. In 2021, clonazolam accounted for approximately one-third of all reported NPS detections but was no longer observed by 2023. Bromazolam first appeared in 2023 and subsequently increased in prevalence, accounting for 25% of detected NPS in 2024 followed by only 10% in 2025. It has yet to be detected in 2026. Delta-8-THC emerged in 2022 and has remained consistently detected since its addition to the testing scope at approximately 30% of detected NPS drugs.

Mitragynine showed stable detection rates through 2021-2025 (approximately 30% of NPS) but was not detected in the first quarter of 2026. This decline coincides with its classification as a controlled substance in Louisiana, effective August 1, 2025.

Among synthetic cannabinoids, MDMB-4en-PINACA was the primary compound identified, with increasing detection frequency each year. Conversely, fluorofentanyl detections steadily declined over the study period, with no cases identified in 2026 to date.

Discussion: This five-year review demonstrates relative stability in the prevalence of commonly encountered drugs, reflecting persistent regional patterns of substance use. In contrast, NPS trends were dynamic and influenced by factors such as analytical scope, market availability, and legislative control.

The largest limitation of this study related to NPS drugs is the scope of testing. Some of the more prevalent NPS drugs seen recently through national trends are not part of the scope of analysis. This is primarily due to unavailability of resources to update current testing panels. The laboratory is in process of implementing a monitoring system for NPS drugs through the TOF screening and available libraries.

Overall, these findings emphasize the importance of maintaining broad-spectrum analytical capabilities and continuously updating testing panels to effectively monitor emerging substances.

Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

P-135

Hidden Administration of 5F-ADB, a Synthetic Cannabinoid, to Minors: Investigation in Blood, Urine, and E-Liquids

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Abstract

Introduction: Case 1: Three underage high school students received an e-cigarette from another high school student. The young people experienced spasms, vomiting, and sickness requiring hospitalization. The laboratory received blood and urine samples from the three subjects, a blood sample from the person who provided the e-cigarettes. The biological samples were collected 12 hours after the incident. Case 2: A middle school student gave an e-cigarette to a classmate. The classmate became ill, suffered from convulsions and vomiting, and was hospitalized. The biological samples were collected 3 hours after the incident.

In both cases, the e-cigarettes and e-liquids were collected during the initial investigations.

Objectives: To present a toxicological strategy to characterize exposure to 5F-ADB (also known as 5F-MDMB-PINACA), a synthetic cannabinoid with rapid metabolism and unestablished chemical stability after time in two different cases. Methods: To characterize the metabolites of 5F-ADB, the drug was incubated at 37°C for 120 minutes with a pool of human liver microsomes (HLMs) and the co-substrates necessary for the function of the main phase 1 (P450) and phase 2 (UGT) enzymes. Analysis of the incubates was performed by LC-HRMS using a Xevo-G2-QTOF-XS (Waters) and revealed three metabolites: O-desmethyl-5F-ADB, 5-OH-pentyl-ADB, and 5-OH-pentyl-O-desmethyl-ADB. After chromatographic (retention time) and spectral (ion) characterization, these metabolites were analyzed in blood and urine specimens extracted with 90/10 hexane/ethyl acetate mixture and hydrolyzed at pH 9.5. A targeted method was developed using LC-MS/MS based on the previously established criteria. The biological samples were analyzed by LC-MS/MS. In parallel, common narcotics, new psychoactive substances (NPS) and drugs were also tested by LC-MS-MS in both cases.

Results: In the first case, the dealer's blood contained THC at 1.2 ng/ml, THC-COOH at 3.3 ng/ml, and a metabolite of 5F-ADB: 5-OH-pentyl-ADB, described in the literature as one of the main metabolite. In the three victims, no metabolites characterized by HLM synthesis were found in both blood or urine. For two of them, blood THC levels exceeded 1 ng/mL. In the second case, the presence of 5F-ADB was confirmed by LC-MS/MS at 0.03 and 0.8 ng/ml in blood and urine of one of the students, respectively, as well as the presence of the metabolite O-Desmethyl 5F-ADB only in the urine described too in the literature as a metabolite. In both cases, LC-HRMS analysis of the e-liquids and the electronic cigarettes demonstrated the presence of 5F-ADB, contaminated with traces of ADB-BUTINACA and MDMB-CHMINACA.

Discussion: In case 1, the lack of any data on the stability of metabolites in biological fluids prevents a definitive conclusion regarding the victims' exposure to 5F-ADB, as the analyses were performed 16 months after the samples were collected although stored at -23°C throughout that time. Case 2 confirms that blood and urine must be collected as close as possible to the moment of consumption. In both cases, the detection of metabolites supports the hypothesis of 5F-ADB exposure. The in vitro production of 5F-ADB metabolites by human liver microsomes is a strategy that can therefore be used in analytical toxicology when the metabolites are not commercially available.

Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

P-136

Analyses of LSD Analogs in Blotter Paper Products Available Online in Japan: Identification of 1EBP-LSD, 1PS-LSD and 1FE-LSD

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Abstract

Introduction: Since around 2010s, products have emerged to contain lysergic acid diethylamide (LSD) analogs as designer drugs. In our previous study, we identified 16 LSD analogs from blotter paper and tablet products distributed in Japan. These include various LSD analogues with acyl groups introduced at the N1 position. It has been suggested that these compounds may be metabolized into LSD in the body and act as prodrugs.

Objectives: To investigate the newly emerged LSD analogs in Japan, we analyzed the compounds contained in blotter paper products labeled as containing "LSD analogs" that were obtained online in 2026.

Methods: Three blotter paper products were obtained in Japan between January 2026 and March 2026. Each blotter paper was cut into 2-mm squares and extracted with methanol for 15 min under sonication. The extracts were concentrated to dryness and redissolved in acetonitrile for gas chromatography–mass spectrometry (GC–MS), liquid chromatography–photodiode array–mass spectrometry (LC–PDA–MS), and LC-hybrid quadrupole time-of-flight MS (LC–QTOF–MS) analyses. For nuclear magnetic resonance (NMR) measurements, each cut paper was sonicated in 1.0 mL of methanol at room temperature for 5 min. The extraction procedure was repeated three times, and the extracts were combined and concentrated by vacuum drying. The residue was dissolved in acetonitrile-d_3 and subjected to NMR analysis using a JEOL JMN-ECZ800 or ECZL800 spectrometer. Compound identification was performed using ^{1}H-NMR, ^{13}C-NMR, heteronuclear multiple quantum coherence (HMQC), heteronuclear multiple-bond correlation (HMBC), H–H correlation spectroscopy (H–H COSY), and nuclear Overhauser effect spectroscopy (NOESY).

Results: Products A and B were labeled as containing "1BP-LSD," while Product C was labeled as containing "1Fe-LSD." NMR analysis identified the compounds present in the three products. Product A contained *N,N*-diethyl-7-methyl-4-(4-(4,4,5,5-tetraethyl-1,3,2-dioxaborolan-2-yl)benzoyl)-4,6,6a,7,8,9-hexahydroindolo[4,3-*fg*]quinoline-9-carboxamide (1EBP-LSD), Product B contained 4-(3-(dimethyl(phenyl)silyl)propanoyl)-*N,N*-diethyl-7-methyl-4,6,6a,7,8,9-hexahydroindolo[4,3-*fg*]quinoline-9-carboxamide (1PS-LSD). Product C contained 4-ferrocenecarbonyl-*N,N*-diethyl-7-methyl-4,6,6a,7,8,9-hexahydroindolo[4,3-*fg*]quinoline-9-carboxamide (1Fe-LSD). All identified compounds were LSD derivatives in which the N1 position was acylated. In blotter paper containing 1PS-LSD, the LC–PDA–MS data revealed the presence of minor compounds, which were estimated to be *iso*-1PS-LSD (the C9-epimerized form of 1PS-LSD).

Discussion: This is the first report of LSD analogs containing boron or iron detected in products distributed in Japan, although silicon-containing analogs such as 1S-LSD and 1SB-LSD have previously been reported. These analogs containing iron, silicon, or boron are likely synthesized to circumvent legal regulations. The carboxylic acids corresponding to the N1 substituents of all three compounds are commercially available as reagents, and N1-acyl derivatives of LSD can be

readily synthesized *via* acid halide intermediates. Furthermore, under standard LC conditions, the retention times of 1EBP-LSD and 1PS-LSD are significantly longer, which may make them difficult to detect in screening analyses using LC. The long retention time of these compounds is thought to indicate high lipophilicity; this high lipophilicity could promote distribution to tissues within the body and delay excretion. Although the specific pharmacological effects of these compounds remain unknown, they may act as prodrugs that are converted to LSD through deacylation in vivo. Further pharmacological studies are necessary to verify this possibility.

Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

Conflict of Interest

Grant/Research Support

P-137

The Rise of Etomidate: Emerging Trends of Veterinary Sedatives and Nitazene Analogues from a National Wastewater Monitoring Campaign in Australia

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Abstract

Introduction: Etomidate, xylazine, and medetomidine are non-opiate sedatives typically used for veterinary applications and have been appearing in the illicit drug market in the United States, Asia, and Europe. Additionally, a class of novel synthetic opioids, nitazenes, have also infiltrated the illicit drug market worldwide. The high potency of these drugs is a public health concern because they can lead to overdose and fatality. Determining the extent of use of these drugs is challenging due to low levels consumed. Wastewater analysis (WWA) is a tool that has been used to monitor population drug consumption trends in near real time. WWA has the potential to be used as an early warning system for the emergence and presence of novel psychoactive substances.

Objectives: The aim of this study was to monitor nitazenes and other sedatives in Australia. A key objective from this data is to develop an early warning system to track the emergence of non-opiate sedatives and nitazenes. The project also examines trends in the occurrence and distribution of these substances over extended periods, providing evidence to support harm reduction initiatives, public health responses, and policy development.

Methods: An analytical method was developed and validated to quantify 25 nitazenes, xylazine, medetomidine and etomidate in wastewater. Solid phase extraction was performed with Xtrackt High Flow CleanScreen DAU Mixed Mode 500mg/6mL columns to yield a 1000-fold concentration factor. A Sciex ExionLC coupled to a Sciex 6500 + QTrap (Ontario, Canada) with a Kinetex 1.7 µm Biphenyl 100Å 150x2.1 mm LC column was used for analysis. Wastewater samples from Australian treatment plants were collected as 24-hour composite samples representing the daily influent wastewater entering the plant. Over 500 wastewater samples covering both weekday and weekend samples from 60 different wastewater treatment plants were analysed between August 2024 and April 2026.

Results: The lower limit of detections for this qualitative method ranged between 0.075 and 0.4 ng/L, depending on the substance. When applied to wastewater samples from across Australia, 19 different nitazenes, xylazine, medetomidine, and etomidate were detected. Xylazine detections remained relatively steady up to a peak in June 2025 (41% of samples) and have been decreasing since. Nitazene detections have also been decreasing in tandem with xylazine, with a peak of 21% of samples; *N*-pyrrolidino etonitazene was the most frequently detected nitazene. Etomidate detections have been increasing since April 2025 when they were introduced to the method, coinciding with the decrease of xylazine and nitazenes.

Discussion: This study has shown that a range of nitazenes and sedatives can be detected in influent wastewater samples in Australia. Solid phase extraction that results in preconcentration of the wastewater sample allows low detection limits, enabling detections in regions of Australia where routine testing for these nitazenes is unavailable. Nearly two years of monitoring revealed that xylazine and nitazenes are in decline, while etomidate prevalence is increasing nationally. In July 2025, China implemented class-wide scheduling of all nitazene-related substances; wastewater monitoring in Australia shows the decline of nitazenes aligns with this scheduling coming into effect.

Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

P-138

Understanding Polydrug Use Patterns Using Networks and Machine Learning Techniques

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Abstract

Introduction: Polydrug use has emerged as a growing public health concern, contributing significantly to overdose deaths, adverse clinical outcomes, and challenges in substance abuse treatment. Traditional statistical approaches often fail to capture complex interrelationships among multiple co-occurring substances. Drug-drug network analysis using Oral Fluid data used in this study offers a powerful framework for modeling and visualizing these interactions, enabling the identification of hidden patterns, highly influential drugs, and evolving community structures within substance use trends.

Objectives: This study aims to investigate patterns of polydrug use among regional samples from the state of Michigan through network-based analysis, identify central and influential substances within the network, and detect the clusters of drug combinations that may indicate emerging trends in polydrug abuse. We aim to identify the temporal evolution of drug prevalence network over the span of the last four years using graph algorithms and association rule mining to uncover latent patterns and inter-drug relationships.

Methods: Oral Fluid specimens analyzed in this study were collected in Michigan through child protective services partners working with our agency and were received between January 1, 2022, and May 30, 2026. The confirmed-positive results for multiple drug classes (amphetamine, methamphetamine, THC, cocaine, opioids, fentanyl, oxycodone, buprenorphine, tramadol) were extracted from the Laboratory Information Management System (LIMS) of Forensic Fluids Laboratories (FFL), Kalamazoo, MI. These data were used to build a drug co-occurrence network (nodes = drugs; edges = co-detections; weights = support/prevalence). Various network metrics such as degree centrality, betweenness centrality, and closeness centrality were computed to identify influential drugs in the network. Drugs that frequently occurred together were studied using the Frequent Pattern Growth algorithm. Temporal trend analysis was conducted to assess changes in the prevalence of specific drug combinations over the period of the study. Visualization and analysis were conducted using Python libraries such as NetworkX, Pandas, and Matplotlib/Seaborn.

Results: The analysis revealed several highly central substances such as delta-9 THC, cocaine, and methamphetamine, indicating their consistent involvement in polydrug combinations. Pairwise prevalence analysis showed that the THC–methamphetamine, THC–fentanyl, and methamphetamine–fentanyl relationships weakened over the study period by 23.68%, 54.47%, and 23.96%, respectively, whereas the THC–cocaine and THC–amphetamine relationships strengthened by 32.58% and 26.61%, respectively. Xylazine and medetomidine appeared in the network in 2023 and 2024, respectively. FP-Growth algorithm identified the most frequent polydrug patterns. Lift analysis consistently found a positive correlation between cocaine and fentanyl from 2022 onward, indicating that the presence of fentanyl increased the likelihood of cocaine also being present rather than reflecting a random coincidence since nearly half of fentanyl-positive patients also tested positive for cocaine.

Discussion: Drug-drug network analysis provides valuable insights into the structural organization and evolution of polydrug use patterns. Identifying influential and bridging substances can support targeted intervention strategies, inform toxicology screening priorities, and aid public health surveillance efforts. Future work may integrate predictive modeling to forecast emerging high-risk combinations. This study demonstrates the utility of networks and unsupervised machine learning techniques in understanding the complexity of substance use behavior and offers a scalable analytical framework for monitoring and mitigating the risks associated with polydrug abuse.

Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

P-139

High-Dimensional Modelling of Forensic Time-Resolved Toxicology Data Using Random Forests and Neural Networks to Infer Cannabinoid-Associated Risk Behaviors

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Abstract

Introduction: Cannabinoids are part of the most popular group of illicit substances in the Western world. The increasing availability of structured, time-resolved forensic data has created new opportunities for multivariate analysis beyond conventional variable-by-variable approaches. However, extracting robust inferential insights, particularly those capturing complex interdependencies between variables, remains challenging.

Objectives: A 5-year study (from 2020 to 2024) is described, with the results of cannabinoid toxicological analyses performed by the Forensic Chemistry and Toxicology Laboratory of the National Institute of Legal Medicine and Forensic Sciences (NILMFS) in the Center of Portugal, with the main objectives: description of the sociodemographic/circumstantial profile; characterization of the results achieved in different contexts [Forensic Clinic (FC), Forensic Pathology (FP) and National Road Safety Authority (NRSA)]; cannabinoids with other psychoactive substances; and implementation/comparison of statistical modelling approaches based on intelligent algorithms, to explore the association between cannabinoid positivity and sociodemographic, circumstantial and toxicological factors.

Methods: Random Forests (RF) and Artificial Neural Networks (ANN) were applied to the cannabinoid positive results achieved over the study period.

Temporal variables included day, month, year, weekday, age and gender. The final set of toxicological predictors included opiates, cocaine and metabolites, amphetamines and related substances, medical substances, ethanol, other volatile substances and carboxyhaemoglobin. Additional binary variables captured circumstantial information derived from medico-legal information (e.g. type of accident, suspected intoxication, trauma, suicide, natural death, *in vivo* vs. post-mortem context).

Results: From the total number of requested analyses on cannabinoids (9532 cases), between January 2020 and December 2024, 881 cases were positive in blood samples after confirmation and quantification of by LC-MS/MS.

The results were separated, with 70.1% (602 cases) from NRSA, 28.1% (241 cases) from FP of the NILMFS and 1.9% (16 cases) from the FC of the NILMFS.

An increasing % of cannabinoid consumption was observed in each year (14.1%, 15.3%, 22.2%, 25.8%, and 22.6%, respectively), with males representing the higher number (91.9%).

With respect to age distribution, the highest consumption was achieved in the 21-30 (38.2%) and 31-40 (22.1%) age groups, corresponding to 60.3% of the cases, indicating that cannabinoid use is concentrated in a relatively young segment of the Portuguese population.

The most frequent co-consumption patterns involved cannabinoids in combination with ethanol (38.5%), cocaine (21.6%), and opiates (18.3%).

Discussion: Consistent associations between cannabinoid positivity, temporal variables, sociodemographic characteristics and co-consumed substances were achieved. While direct model fitting primarily reflects temporal covariation, predictive validation indicates interpretable relationships between polysubstance use and high-risk contexts.

The predominance of young males and the frequent co-occurrence of cannabinoids with ethanol, cocaine, opioids, and benzodiazepines indicate that cannabinoid positivity is often part of broader risk behaviours, especially in contexts with potential legal or clinical consequences, also suggesting that cannabinoid use frequently occurs with other substances increasing impairment, violence, trauma, and overdose risk. The comparison between RF and ANN also highlights that model performance depends on sample size, class imbalance, and the type of outcome under study.

Differences between direct fitting and predictive validation underline that apparently strong fitted models should not be overinterpreted. In this study, predictive models were generally more useful for identifying interpretable associations, whereas fitting models tended to emphasize temporal covariation.

Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

P-140

Evaluation of an Integrated LC–MS Quantitation Workflow for Forensic Toxicology Applications

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Abstract

Introduction: Forensic toxicology laboratories require quantitative LC–MS workflows that are reproducible, efficient, and defensible under accreditation and legal scrutiny. Challenges commonly include manual data handling between acquisition and quantitation platforms, inconsistent method setup, and the significant analyst time required for chromatographic review of large multi-analyte datasets. Integrated software environments that consolidate acquisition, optimization, data processing, and review may address these limitations while supporting data integrity requirements.

Objectives: The objective of this work was to evaluate the applicability of the waters_connect™ platform with MS Quan for routine screening and quantitative LC–MS workflows typical of forensic toxicology laboratories.

Methods: Representative qualitative and quantitative multi-analyte assays were acquired and processed using waters_connect with MS Quan. Method development, including automated retention time updating for 100's of analytes, MRM optimization via infusion, automated source optimization, data acquisition, data processing, and review were conducted within the same software environment. Data were assessed using a combination of full manual review and configurable exception-focused review rules reflecting commonly used forensic acceptance criteria (e.g., ion ratios, retention time tolerance, signal-to-noise thresholds, blanks, internal standards, and Calibration/QC performance).

Results: The quantitative dataset comprises 98 analytes, each monitored using 2–3 MRM transitions, along with their corresponding isotopically labeled internal standards (1–2 MRM transitions each). Processing a sequence of 55 samples - corresponding to approximately 25,000 chromatograms - is completed in under 60 seconds. The comprehensive overview and the exception focused review allow for the assessment of linearity and concentration deviations across all 588 calibrators and 392 QC samples in only a few minutes, resulting in a reduction of manual interactions by more than 90%. Restricting data review to flagged peak integrations further decreases total processing time by approximately 50%. Additionally, data inspection by injection, with analytes organized according to compound class, facilitates the identification of relationships between parent compounds and their metabolites.

Discussion: End-to-end integration of system control, acquisition, quantitation, and reporting eliminated intermediate data transfer steps between software modules, reducing opportunities for transcription and handling errors. Centralized access to instrument tuning and MRM optimization data facilitated consistent method development and transfer, with retention times and dwell times automatically incorporated into acquisition methods.

The workflow-based data processing approach in MS Quan enabled structured review of large datasets containing numerous analytes. Exception-focused review rules automatically highlighted results outside predefined acceptance criteria, allowing analysts to prioritize potential issues while maintaining access to full chromatographic inspection where required. Visualization tools supporting simultaneous review of multiple MRM traces aided rapid identification of carryover, integration, or matrix-related effects across batches.

Automated system suitability assessments (e.g., retention time stability, peak response, ion ratios, and peak shape metrics) provided documented verification of instrument performance prior to case sample analysis. Software features supporting version control, audit trails, user access management, and traceability of analytical changes were available throughout acquisition and processing, aligning with expectations for accredited forensic laboratories.

The integrated waters_connect and MS Quan workflow supported efficient and defensible quantitative LC–MS analysis for forensic toxicology applications. Consolidation of acquisition, optimization, processing, and review within a single environment reduced manual handling.

Disclosure

Yes, I, or a member of my immediate family, has a financial interest to disclose.

Conflict of Interest

I am a paid employee of Waters Corporation.

P-141

Retro-BAC: An R Shiny Application for Retrograde Extrapolation Alcohol Calculation With IA Chatbot

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Abstract

Introduction: Retrograde extrapolation alcohol calculation is frequently requested by attorneys to forensic toxicology laboratories. In these cases, the blood alcohol concentration (BAC) is used as a reference value. In 2018, experts called for creating consensus standards for conducting retrograde extrapolations of BAC to guarantee a scientific foundation for such estimates and their usage in judicial scenarios, and, in response to this request in 2024, guidance was issued. Currently using programming languages tools are created to support work in the forensic area, and IA Chatbots are GPT-based no-code platforms that enable custom chatbots trained on guidelines, offering intelligent NLP-driven assistance, 24/7 support, and seamless integration into forensic science applications to enhance user experience and operational efficiency.

Objectives: The objective was to develop an application in R Shiny with an IA Chatbot for the retrograde extrapolation alcohol calculation and application on real cases.

Methods: To develop the application for the retrograde extrapolation calculation alcohol, the open-source and accessible R software was used. The integrated development environment used was RStudio, and RShiny was used to create the application. In addition, multiple packages were incorporated to create the script, such as shiny, ggplot2, rmarkdown, and others. An AI Chatbot was added to handle inquiries based on the guidelines used in the app's development. Calculations were performed following the ANSI/ASB Best Practice Recommendation 122, 1st Ed. 2024 guidelines. The calculation shall be performed using a range of elimination rate: 0.10 - 0.25 g/L/h; encompasses the majority of the population regardless of age, sex, ethnicity, and drinking experience. The basic calculation for retrograde extrapolation is expressed as:

$$AC_{inc} = AC_{test} + (\beta \times T)$$

AC_{inc} : estimated alcohol concentration at the time of the incident (g/L).

AC_{test} : measured alcohol concentration(g/L)

β : elimination rate (g/L/h)

T : time between incident and time of blood draw / breath test (hours).

Tests were carried out with the results of post-absorptive real cases.

Results: When using the application in real cases, it was observed that its tool calculates the alcohol range of the individual using an alcohol concentration at a given point in time and estimates what the concentration would have been at an earlier time based on the BAC value and the times reported in the case background. The calculations performed by the tool are visualized in real time, by applying the white box concept. In addition, the application provides a graph explaining the timeline of BAC of the case, and a downloadable report with estimated information serves as registration documentation. In addition, queries were made through the AI Chatbot to the guideline based on the results obtained.

Discussion: The Retro-BAC open-access application developed in RShiny for the retrograde extrapolation calculation provides the alcohol range in subjects post-absorptive, permits a friendly environment for results, and helps to deliver relevant information based on a consensus and updated guideline, and queries regarding the results were made through an AI chatbot to better interpret them. The application allows to generate automatic word reports, this report must always be reviewed by an expert before being submitted to the attorneys. The next step for this tool will be to have a Spanish-language version to increase its scope.

Retro-BAC App: https://forensictox.shinyapps.io/Retro-BAC/

Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

P-142

Postmortem Toxicology Trends in Pediatric Decedents aged 6 Years and under: A Retrospective STORM Surveillance Analysis

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Abstract

Introduction: Postmortem toxicology is often essential in pediatric death investigations for cause of death determinations. Typically, toxicology results in young children reflect therapeutic drug administration or substance exposure. However, rare cases of illicit or multiple atypical substance exposure can occur. Drug surveillance programs such as the Swift Toxicology of Overdose Related Mortalities (STORM) may provide valuable information regarding drug exposure and emerging trends in this vulnerable group. This study aims to present findings from STORM for pediatric decedents ≤6 years.

Objectives: Characterize substances detected, toxicological patterns, and emerging trends in postmortem cases of children ≤6 years of age.

Methods: A retrospective review of pediatric postmortem blood samples (PB) collected through the STORM program from January 2017 to April 10, 2026, was conducted. Toxicological results were assessed for novel substances, illicit drugs, recurring patterns of exposure, and polydrug combinations. Temporal and geographical trends were also assessed. Pediatric cases from STORM were analyzed using a solid-phase extraction (SPE) technique with Clean Screen XCEL-I Extraction Columns (UCT PN ZSXE010-D), followed by liquid chromatography–tandem mass spectrometry (LC-MS/MS) analysis, which used Water Acquity for LC and Waters TQD for MS/MS. Deuterated internal standards were added to samples and quality control material, then extracted. SPE extracts were dried, reconstituted, and analyzed via LC-MS/MS. Chromatographic separation was accomplished via a bi-phenyl column at 40°C, with electrospray ionization in positive mode. Two transitions were monitored per analyte. The Restek Guard Column: Raptor Bi-Phenyl EXP and Restek Column: Raptor Bi-Phenyl with sizes 5 mm, 2.1 mm, and 2.7 µm were used. Retention time, transition mass, and peak area ratios were identified and reported.

Results: 195 pediatric postmortem cases (≤6 years old) were identified, with opioids being a common occurrence. Fentanyl was found in 16 cases, 11 showing active metabolism through the detection of norfentanyl. The fentanyl analog, para-fluorofentanyl (n=2) and 4-ANPP (n=2) were also detected, indicating possible illicit drug exposure. Other substances included heroin (n=2), confirmed by 6-acetylmorphine, methamphetamine (n=2), morphine (n=7), acetyl fentanyl (n=4), xylazine (n=2), and cannabinoids (n=2). Children positive for fentanyl were 1 to 6 years old (average = 2.1 years). Cocaine-positive cases (n=3) involved children under 1 year old. Polydrug exposure was not uncommon, with notable cases exhibiting fentanyl analogs, stimulants, and opioids. As an example, one case was a 4-year-old having postmortem blood results of fentanyl, 4-ANPP, acetyl fentanyl, norfentanyl, para-fluorofentanyl, amphetamine, and methamphetamine. In two unrelated cases of <1-year-olds, 6-acetylmorphine, codeine, fentanyl, morphine, para-fluorofentanyl, xylazine, and heroin were detected in the decedent's postmortem blood (PB). 6-acetylmorphine, morphine, gabapentin, and heroin were detected in the PB of the other <1-year-old.

Discussion: This study demonstrates that postmortem toxicology can detect new substances and emerging drug patterns. The presence of polydrug use indicates complex exposures needing a high degree of scrutiny. Finding cocaine in two infants under 1 year old, with geographic proximity, suggests localized exposure. These results highlight the importance of comprehensive testing and surveillance programs like STORM to identify patterns and improve public health actions to protect vulnerable groups.

Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

P-143

Systemic Distribution of Tolfenpyrad and its Major Metabolite PTCA in a Case of Fatal Poisoning

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Abstract

Introduction: Tolfenpyrad (TFP) is a pyrazole-carboxamide insecticide developed in Japan; it inhibits the mitochondrial complex I of the electron transport chain. Animal studies conducted by the manufacturer and a human hepatocyte-based *in vitro* study reported by Jiang et al. (2024) demonstrated that TFP is biotransformed into its major oxidative metabolite, 4-[4-[(4-chloro-3-ethyl-1-methylpyrazol-5-yl)carbonylaminomethyl]phenoxy]benzoic acid (PTCA). However, no studies have demonstrated the systemic distribution of TFP and PTCA in humans.

Objectives: This study aimed to quantify TFP and PTCA in blood, body fluids, and multiple organs in a fatal case of acute TFP ingestion and to characterize their human *in vivo* distribution.

Methods: Blood, liver, bile, kidney, adipose tissue, brain, vitreous humor, and gastric contents were analyzed. After protein precipitation with acetonitrile, TFP and PTCA were quantified using LC–MS/MS in MRM mode. Tebufenpyrad, a structural analog of TFP, was used as an internal standard. TFP level in the blood was quantified using an external calibration curve. PTCA in the blood, and both TFP and PTCA in all other biological samples were quantified using the standard addition method. The accuracy and precision of each quantification approach met the required analytical criteria.

Results: The liver exhibited the highest concentrations of both compounds (3.3 μg/g TFP and 2.3 μg/g PTCA). The bile sample also contained a high concentration of PTCA (2.2 μg/mL). PTCA levels exceeded those of TFP in all blood, fluid, and organ samples, except for the liver and gastric contents, with particularly high levels in the kidney (2.0 μg/g).

Discussion: The high concentrations of the analytes in the liver and bile suggest that human hepatic metabolism and biliary excretion constitute the major pathways of TFP disposition. The finding that PTCA exceeds TFP in most matrices indicates that TFP is rapidly oxidized *in vivo* and circulates predominantly in the form of PTCA. Although the urine sample was unavailable, the elevated renal PTCA concentration supported the urinary excretion pathway previously reported in animal studies.

This is the first report demonstrating the post-ingestion metabolism, distribution, and excretion of TFP in human organs, supported by *in vivo* human data consistent with metabolic pathways previously proposed in animal and in vitro studies. Furthermore, the predominance of PTCA is an important finding that supports its potential utility as a monitoring marker in future assessments.

Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

P-144

Validated LC–MS/MS Quantification and Assessment of Postmortem Redistribution of Mirogabalin in a Fatal Multidrug Intoxication Case

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Abstract

Introduction: Mirogabalin, a gabapentinoid α2δ ligand of voltage-gated calcium channels, is an analgesic prescribed for peripheral neuropathic pain. Although clinical pharmacokinetic data are available, postmortem toxicological data on mirogabalin remain limited, and its postmortem redistribution has not been evaluated in fatal mirogabalin-related cases.

Objectives: This study aimed to establish and validate an analytical method for quantifying mirogabalin in postmortem blood and urine from a fatal multidrug intoxication case using LC–MS/MS. Postmortem redistribution was assessed using right heart-to-femoral blood and liver-to-femoral blood concentration ratios.

Methods: A man in his 60s was found dead at home. Empty blister packs at the scene corresponded to possible maximum available doses of 650 mg mirogabalin besilate and 3000 mg sertraline hydrochloride. A forensic autopsy was performed 6 days after death. Mirogabalin was extracted using the QuEChERS method, purified with Captiva ND Lipids cartridges, and analyzed by LC–MS/MS. The method was validated in blood and urine for calibration linearity, accuracy, precision, matrix effects, recovery, and stability over 7 days. Femoral blood and liver specimens from the same case were additionally analyzed to calculate right heart-to-femoral blood and liver-to-femoral blood concentration ratios.

Results: Correlation coefficient values for calibration linearity from 0.2 to 100 μg/mL were 0.9972 in blood and 0.9943 in urine, respectively. Accuracy, expressed as percent relative error, ranged from –13.6 to 14.6% in blood and from –2.2 to 13.6% in urine. Precision, expressed as percent coefficient of variation, ranged from 1.5 to 11.8% in blood and from 4.6 to 11.8% in urine. Matrix effects were 51.3–69.9% in blood and 36.1–67.0% in urine, indicating ion suppression. Recovery rates were 69.5–80.8% in blood and 72.2–97.4% in urine. Stability over 7 days in spiked specimens was 80.0–116.5% in blood and 66.6–98.6% in urine at 22 °C, 4 °C, and –80 °C. Mirogabalin concentrations were 1.99 μg/mL in right heart blood, 1.39 μg/mL in femoral blood, 3.56 μg/mL in urine, and 2.39 μg/g in liver. The right heart-to-femoral blood ratio was 1.43, and the liver-to-femoral blood ratio was 1.71. Sertraline was quantified in right heart blood at 0.493 μg/mL. Ethanol concentrations were 0.133 mg/mL in right heart blood and 0.329 mg/mL in left heart blood. A urine immunoassay screening test was positive for benzodiazepines, whereas no specific benzodiazepine was confirmed in blood.

Discussion: The LC–MS/MS method validated in blood and urine supported toxicological interpretation of the measured mirogabalin concentrations in this fatal multidrug intoxication case, and additional femoral blood and liver concentration data were used for postmortem redistribution assessment. The right heart-to-femoral blood ratio of 1.43 and liver-to-femoral blood ratio of 1.71 indicated postmortem redistribution of mirogabalin to be limited, consistent with previous reports for gabapentin and pregabalin. The cause of death was interpreted as multidrug intoxication

involving sertraline, mirogabalin, ethanol, and benzodiazepine exposure. These concentration data and postmortem redistribution ratios provide reference information for future forensic interpretation of suspected mirogabalin-related deaths.

Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

P-146

Perimortem-Postmortem Drug Concentration Discrepancies in a Fatal Pentobarbital Intoxication

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Abstract

Introduction: Interpretation of drug concentrations in postmortem toxicology is complicated by postmortem redistribution (PMR), while peri- and postmortem absorption-like processes may additionally contribute to concentration changes after death. Discrepancies between antemortem and postmortem values may lead to significant overestimation of drug exposure at the time of death. We report a fatal pentobarbital intoxication case, showing marked postmortem concentration increases and a misleading segmental hair profile.

Objectives: In this study, we compare perimortem and postmortem drug concentrations across matrices; evaluate contribution of peri and postmortem absorption-like processes and PMR; assess the interpretative value of segmental hair analysis.

Methods: A 41-year-old male was found in cardiocirculatory arrest in a supine position on his bed. At the scene, two empty glass bottles (labeled as (1) pentobarbital for veterinary use and (2) a skin cosmetic product), and empty zolpidem blister packs were present. Perimortem blood was sampled in a hospital setting during cardiocirculatory arrest, approximately 30 minutes pre-death declaration. A forensic autopsy was performed 7 days later. Postmortem specimens included femoral and cardiac blood, vitreous humor, urine, brain, liver, kidney, lung, gastric content, and esophageal content. Segmental hair analysis was performed using 20 consecutive 1-cm segments (proximal S1 to distal S20) . All results were achieved using fully validated LC–MS/MS methods.

Results: Perimortem concentrations were consistent across matrices, with pentobarbital ranging from 32 to 37 µg/mL and zolpidem from 6 to 12 ng/mL. Postmortem concentrations were markedly increased: femoral blood showed 162 µg/mL (pentobarbital) and 1400 ng/mL (zolpidem), while cardiac blood reached 206 µg/mL and 2500 ng/mL, respectively. Central-to-peripheral ratios were moderate (≈1.3–1.8). Vitreous humor concentrations were lower (22 µg/mL (pentobarbital) and 9 ng/mL (zolpidem). Extremely high concentrations were detected in gastric and esophageal contents, indicating a large unabsorbed drug reservoir. Tissue concentrations were elevated, particularly in liver and lung. Segmental hair analysis revealed detectable concentrations of both compounds across all segments. A similar distribution was observed in both hair-washing solution and hair samples, with relatively low concentrations in proximal segments (S1–S10).

Discussion: Perimortem sampling suggests that active gastrointestinal absorption appeared to be negligible at that time. The marked increase in postmortem concentrations, including in femoral blood (≈5x for pentobarbital and ≈150x for zolpidem) is unlikely to be fully explained by PMR alone, according to literature. These findings may support a combined effect of (i) a rising concentration phase prior to arrest, (ii) passive diffusion from a large gastroesophageal reservoir after death,

and (iii) redistribution from highly perfused tissues, particularly for pentobarbital. Vitreous humor concentrations likely better reflect late perimortem blood levels, highlighting the risk of overestimation when relying solely on postmortem blood. The segmental hair pattern is more consistent with peri- and postmortem external contamination (washing/hair ratio >0.5) resulting in a spatial distribution that may be misinterpreted as temporal exposure. This case demonstrates that postmortem blood (even peripheral) concentrations may substantially exceed perimortem levels due to combined peri- and postmortem absorption-like processes and PMR. Segmental hair analysis should be cautiously interpreted in such contexts. Integrated interpretation of perimortem blood, unconventional matrices (i.e. vitreous humor) is essential for accurate forensic conclusions.

Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

P-147

Illicit Drug-Related Deaths in Singapore

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Abstract

Introduction: Illicit drugs remain a significant contributor to mortality in forensic casework worldwide. Data on drug-related deaths provide valuable insights into evolving drug trends and patterns of polydrug use. Post-mortem toxicology plays a critical role in identifying substances implicated in fatal intoxications and in supporting medico-legal investigations.

Objectives: This study aims to review illicit drug-related deaths in Singapore and to describe the demographic characteristics, commonly detected illicit drugs, patterns of co-detection, and circumstances of death.

Methods: A retrospective review was conducted on post-mortem cases received by our laboratory from 2024 to 2025. Cases were classified as illicit drug-related deaths when one or more illicit drugs were detected in post-mortem blood specimens. Urine toxicology findings were also reviewed as a complementary matrix to assess broader patterns of drug use. Biological specimens were analysed using validated screening and confirmatory methods, including liquid chromatography–high resolution mass spectrometry (LC-HRMS) and liquid chromatography–ion trap mass spectrometry (LC-IT/MS).

Results: Preliminary analysis identified 156 illicit drug-related fatalities, comprising 126 males and 30 females. These cases accounted for approximately 6% of the 2,425 post-mortem cases received over the two-year period, with a 60% increase observed in 2025 (96 cases) compared to 2024 (60 cases). Methamphetamine, etomidate, and MDMA were the most commonly detected illicit drugs, with methamphetamine identified in 65% of cases. Polydrug use was common, with 75 cases involving two or more drugs, and six cases involving four or more illicit substances. Novel psychoactive substances (2-fluoro-2-oxo-PCE, 4-CMC, and 4-MMC) were detected in six cases.

The most common circumstance of death was sudden death (57%). Among these, six cases involved the use of gamma-hydroxybutyric acid (GHB) in combination with either MDMA or methamphetamine. Other circumstances included falls from height (29%) and road traffic accidents (13%). Among the fatalities, 65% of females and 50% of males were below the age of 40.

Discussion: This review provides an overview of illicit drug-related deaths in Singapore, with a notable increase in cases in 2025 and a predominance of younger individuals among the fatalities. The high prevalence of polydrug use underscores the importance of comprehensive toxicological screening and careful interpretation of combined drug effects in fatal cases. While blood toxicology remains central to assessing the contribution of illicit drugs to death, urine analysis offers additional insights into broader patterns of drug exposure.

Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

P-148

Atypical Fatality in Postpartum Depression: Fatal Synergism of Alcohols, CNS Depressants, and Dissociative Anesthetic

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Abstract

Introduction: Maternal deaths during the immediate postpartum period are commonly attributed to obstetric or anesthetic complications; however, toxicological contributions may be underrecognized. Postpartum depression is a significant public health concern associated with an increased risk of intentional or unintentional polysubstance ingestion. Interpretation becomes particularly challenging when multiple central nervous system (CNS) depressants are detected at individually sublethal or therapeutic concentrations. A 27-year-old female with a history of recent cesarean delivery and postpartum depression was found unresponsive after a domestic conflict, raising serious forensic concerns. An autopsy was performed, and biological samples collected for toxicological and histopathological examination.

Objectives: To highlight the interpretative challenges associated with polysubstance ingestion in postpartum depression, emphasizing synergistic toxicodynamic interactions and clinicopathological correlation in forensic death investigation.

Methods: Postmortem (peripheral blood, urine and gastric contents) samples were analyzed using comprehensive toxicological analysis. Volatile compounds were quantified via headspace GC-FID, while drugs were identified and quantified using GC-MS via Scan and SIM modes, respectively.

Results: Autopsy revealed pulmonary petechiae, consistent with hypoxia. Histopathological examination demonstrated hepatocellular injury, renal tubular degeneration, pulmonary edema, and brain congestion. Toxicological analysis of blood quantified methanol (0.034 g/dL), ethanol (0.076 g/dL), diazepam (0.05 mg/L) and ketamine (0.05 mg/L). Urine analysis confirmed methanol (0.05 g/dL) and metronidazole.

Toxicological findings were interpreted in correlation with clinical history, autopsy, analytical quantification and histopathological results, alongside known pharmacokinetic and toxicodynamic data.

Discussion: This case study demonstrates the synergistic CNS depression despite sub-lethal individual drug levels. Reported lethal concentrations (ethanol >0.3 – 0.4 g/dL, methanol >0.2 g/dL, ketamine rarely fatal alone and diazepam fatal at very high levels usually) are well above the detected concentrations [1]. However, combined presence of ethanol with CNS depressants indicates significant pharmacodynamic interaction.

Although the detected concentrations were individually below commonly reported lethal ranges, the combined presence of ethanol, diazepam, ketamine and methanol supports significant synergistic CNS and respiratory depression. Ethanol potentiates GABAergic inhibition and enhances benzodiazepine-mediated sedation, while ketamine contributes additional NMDA receptor antagonism and dissociative anesthetic effects. Concurrent methanol exposure may further exacerbate tissue hypoxia and metabolic compromise.

The combined pharmacodynamic effects likely resulted in profound neuro-respiratory depression, impaired airway protective reflexes and fatal hypoxic injury. Histopathological findings correlated with toxicological evidence of respiratory and CNS depression [1, 4]. This case highlights the limitations of interpreting postmortem toxicology solely through numerical concentration thresholds. In vulnerable postpartum populations complicated by psychological stress and possible substance misuse, comprehensive clinicopathological correlation remains essential for accurate forensic interpretation.

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Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

P-149

Determination of Hypophosphite in Blood by LC-MS/MS for the Confirmation of Phosphine Poisoning

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Abstract

Introduction: Aluminum phosphide is widely used as a fumigant pesticide for stored grains and dried food products. Upon exposure to moisture or gastric acid, it rapidly decomposes to release phosphine gas (PH_3), a highly toxic compound responsible for many accidental and intentional poisonings worldwide. Phosphine poisoning is associated with high mortality due to rapid systemic toxicity, making timely confirmation critical in forensic investigations. After absorption, phosphine is oxidized to hypophosphite, phosphite, and ultimately phosphate. Among these, hypophosphite is absent in normal human blood, making it a specific and valuable biomarker for phosphine exposure.

Objectives: This study aimed to develop and validate a rapid, sensitive, and reliable LC–MS/MS method for determining hypophosphite in human blood to support confirmation of aluminum phosphide and phosphine poisoning cases.

Methods: Whole blood samples (0.2 mL) underwent protein precipitation with acetonitrile followed by phase separation using dichloromethane. The aqueous layer was filtered and analyzed directly. Chromatographic separation employed hydrophilic interaction liquid chromatography with an aqueous ammonium formate buffer (mobile phase A) and acetonitrile (mobile phase B). The gradient began at 8% A/92% B (0–0.5 min), increased linearly to 50% A/50% B at 4.0 min, held until 6.0 min, then returned to initial conditions at 6.1 min and equilibrated until 8.0 min. Detection used electrospray ionization in negative mode with multiple reaction monitoring. Matrix-matched calibration curves were prepared using blank human blood, with six replicates analyzed at each concentration level. Only intra-batch precision was evaluated. Method performance was assessed for linearity, recovery, precision, selectivity, and applicability to real forensic cases. Freeze–thaw stability and long-term sample stability were not evaluated.

Results: The method demonstrated excellent linearity from 5–200 ng/mL, with correlation coefficients exceeding 0.9995. Extraction recoveries across six blood matrices ranged from 70% to 135%, with relative standard deviations below 10%, indicating acceptable precision and robustness despite matrix variability. Comparisons between blank and spiked matrices showed no significant interferences. The analytical workflow enabled rapid processing, with chromatographic separation completed in 8 minutes per injection. Application to two confirmed poisoning cases yielded hypophosphite concentrations of 107.8 ng/mL and 119.9 ng/mL, consistent with reported clinical levels ranging from tens to hundreds of ppb following aluminum phosphide exposure.

Discussion: This study presents a practical LC–MS/MS method for determining hypophosphite in blood as a specific biomarker of phosphine exposure. The method combines straightforward sample preparation with rapid analysis and sufficient sensitivity for forensic applications. Successful application to authentic poisoning cases demonstrates its utility for confirming aluminum phosphide intoxication, particularly when direct phosphine detection is not feasible. Overall, the method provides a valuable analytical tool for toxicology laboratories investigating phosphorus-based poisoning events.

Disclosure

Yes, I, or a member of my immediate family, has a financial interest to disclose.

Conflict of Interest

Salary from company at which its products are being discussed

P-150

A Tale of Two Barbs: Suicidal Ingestion of Barbiturates in Two Unique Cases in Miami, FL

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Abstract

Introduction: The Miami-Dade Medical Examiner Department (MDME) recently received two unusual cases, that involved suicidal ingestions of barbiturates.

Case 1 involved a 91-year-old Asian male found unresponsive in his bed by his wife. A suicide note was found written in Mandarin on old MDME letterhead. An antique amber bottle of laboratory grade Sodium Barbital manufactured by Fisher Laboratory Chemical and a cup containing an unknown white substance was found nearby.

Case 2 involved a 55-year-old white female found sitting unresponsive slumped over the kitchen table with a suicide note underneath her head and a second one inside a bag. There was a partially emptied bottle of tequila and cups containing fluid on the table next to her. During MDME scene response, a search of the trash revealed two empty bottles of a facial cleansing product.

Objectives: To demonstrate the value of thorough scene investigation and the importance of including barbiturates in the scope of testing.

Methods: Biological specimens were screened for volatiles utilizing GC-FID-Headspace. Screening for basic and acid/neutral drugs was performed utilizing GC-MS, LC-MS/MS and LC-IonTrapMS^n. Quantitation of barbiturates was performed utilizing supported liquid extraction (SLE) followed by LC-MS/MS. Quantitation of antihistamines was performed by solid phase extraction (SPE) followed by analysis by LC-MS/MS, and quantitation of oxycodone was performed utilizing SPE followed by analysis by GC-MS/MS. Gastric and small intestinal contents were quantitated by method of standard addition.

Results:

Case 1:

	Iliac Vein Blood	**Gastric Contents**
Barbital	473 mg/L	579 mg total

Atorvastatin, cetirizine, norchlorcyclizine, clopidogrel, acetaminophen, losartan and metoprolol were also detected qualitatively.

Case 2:

	Iliac Vein Blood	Gastric Contents	Small Intestinal Contents	Ocular Fluid
Pentobarbital	50 mg/L	641 mg total	3189 mg total	
Ethanol	0.092 g/dL			0.043 g/dL
Diphenhydramine	0.227 mg/L	0.029 mg total	0.476 mg total	
Hydroxyzine	0.062 mg/L	Detected	Detected	
Oxycodone	Undetected	2.1 mg total	0.009 mg total	

Cetirizine, norchlorcyclizine and labetalol were also detected qualitatively. In addition, fluids from a water bottle and a facial cleanser bottle were found to contain pentobarbital.

Discussion: Case 1 had worked as a technician at the MDME several decades previously. It is hypothesized that he may have obtained the bottle of sodium barbital at this time, as well as the letterhead on which he wrote his suicide note.

It appears that case 2 likely purchased the pentobarbital on the internet, as a search revealed that the facial cleansing product from the scene “Natura Skin Cleanser – Face Masque” is sold as a disguised euthanasia agent.

The cause of death in case 1 was ruled “Acute Barbital Toxicity” with the manner “Suicide”. The cause of death in case 2 was ruled “Acute Combined Drug Toxicity (Pentobarbital, Oxycodone, Diphenhydramine, Ethanol)” and the manner “Suicide”.

These two cases highlight the need for toxicology laboratories to have a broad scope of testing, since uncommon drugs may be detected. It is also imperative to have good scene investigation, and the ability to test pertinent evidence that may be found in relation to a death investigation to provide accurate cause and manner of death.

Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

P-151

Ethanol Distribution Across Blood, Vitreous Humor, and Urine: Associations with Manner of Death and Physiological State

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Abstract

Introduction: Interpretation of postmortem ethanol concentrations remains a persistent challenge in forensic toxicology due to variability across biological matrices and changing physiological conditions. Although vitreous humor is considered a more stable matrix than blood, reliance on single-matrix interpretation remains limited. A multimatrix approach may provide a more physiologically meaningful framework.

Objectives: To evaluate ethanol distribution across blood, vitreous humor, and urine, and to determine whether multimatrix patterns reflect underlying physiological phases and differ according to manner of death.

Methods: A retrospective study was conducted on forensic cases from the Institute of Legal Medicine of Galicia (IMELGA), analyzed at the Forensic Toxicology Laboratory of the University of Santiago de Compostela (Spain) between 2015 and 2025. Cases with paired blood and vitreous humor ethanol concentrations (n = 272) were included. A subset of these case (n = 97) also had urine ethanol concentrations available, enabling direct multimatrix comparisons across blood, vitreous humor, and urine, including combined VH/B and U/B ratio analyses. The study population comprised 227 males and 45 females, with ages ranging from 16 to 92 years. Blood ethanol concentrations were determined using gas chromatography with flame ionization detection (GC-FID). Vitreous humor-to-blood (VH/B) and urine-to-blood (U/B) ratios were calculated and categorized into predefined ranges reflecting physiological phases (absorptive, equilibrium, post-absorptive), further subdivided to improve analytical resolution. Cases were stratified by manner of death. Statistical analyses included chi-square tests, Cramér's V, Kruskal–Wallis tests, and post-hoc comparisons. Unsupervised K-means clustering was applied for exploratory multimatrix analysis.

Results: VH/B distributions differed significantly across manners of death ($p < 0.05$), primarily driven by variation between accidental and natural cases. Accidental deaths were predominantly associated with equilibrium ranges (VH/B 0.80–1.14), whereas natural deaths showed a broader distribution with increased representation of higher ratios (≥1.30), consistent with post-absorptive conditions. Suicidal cases exhibited a heterogeneous pattern. Median VH/B values were similar across groups, although dispersion differed, particularly in natural deaths. Urine-to-blood ratios alone showed high variability, but when interpreted alongside VH/B they helped identify concordant and discordant patterns. Clustering analysis revealed a dominant central distribution with a limited number of extreme cases, supporting a continuous distribution model. No clear age-dependent trend was observed, although greater variability was noted in older individuals, particularly in natural deaths.

Discussion: Ethanol distribution across biological matrices reflects a continuous physiological spectrum rather than discrete categories. While vitreous humor provides a stable reference for postmortem ethanol assessment, urine contributes complementary information, particularly in the identification of discordant cases. Age-related variability, particularly in natural deaths, may reflect underlying physiological changes affecting ethanol distribution. The integration of multiple matrices allows identification of patterns not evident in single-matrix analysis and may enhance the contextual interpretation of ethanol findings in forensic casework.

Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

P-152

Postmortem Drug and Alcohol Detection Among Deceased Foreign Nationals in Bangkok, Thailand (2024–2025)

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Abstract

Introduction: Deaths among foreign nationals in Thailand represent an important forensic and public health concern, particularly in Bangkok, a major international hub. However, postmortem toxicological data describing substance exposure patterns among foreign decedents remain limited. Characterizing drug and alcohol detection patterns may improve understanding of substance exposure profiles and support interpretation of postmortem toxicology findings.

Objective: This study aimed to characterize demographic characteristics and postmortem drug and alcohol detection patterns among foreign nationals who died in Bangkok, Thailand, and to compare toxicological findings between 2024 and 2025, including variations by region of origin.

Method: A retrospective study was conducted using postmortem toxicology data from foreign nationals examined at the Toxicology Laboratory, King Chulalongkorn Memorial Hospital, Thai Red Cross Society, Bangkok, Thailand, between 1 January 2024 and 31 December 2025. Demographic characteristics, nationality distribution, and major drug class detection patterns were analyzed and compared between study years. Alcohol co-detection patterns and overall drug detection profiles were also evaluated. Variations by region of origin were assessed using chi-square tests and Fisher's exact tests, where appropriate. Odds ratios (ORs) were calculated for year-to-year comparisons.

Results: A total of 388 foreign decedents were included (208 cases in 2024 and 180 cases in 2025). Most decedents were male in both years (84.1% and 86.7%), with median ages of 46 and 47 years, respectively. Myanmar nationals represented the largest proportion of cases in both study years. Alcohol was detected in 18.8% of cases in 2024 and 22.2% in 2025. Significant increases were observed in stimulant detection (12.0% vs. 19.4%, OR = 1.77, $p = 0.040$) and kratom alkaloid detection (4.8% vs. 11.1%, OR = 2.48, $p = 0.020$). Among alcohol-positive cases, co-detection with other drugs increased significantly in 2025 ($p = 0.027$). Overall drug detection profiles differed significantly between study years ($p = 0.014$), characterized by fewer cases without drug detection and a higher prevalence of single-drug detections in 2025. When data from both years were combined, significant variations by region of origin were observed. Kratom alkaloid detection was significantly more common among decedents from the Association of Southeast Asian Nations (ASEAN) region ($p < 0.001$), whereas opioid and benzodiazepine detections were more frequent among decedents from Europe and the Americas ($p = 0.001$ and $p = 0.040$, respectively). Alcohol detection also differed significantly across regions ($p = 0.030$).

Discussion: Postmortem toxicological findings among foreign decedents in Bangkok demonstrated frequent detection of alcohol, stimulants, opioids, benzodiazepines, and kratom alkaloids. Compared with 2024, 2025 showed increased detection of stimulants and kratom alkaloids, as well as greater co-detection of alcohol with other substances. Significant variations by region of origin suggest distinct substance exposure profiles among different

foreign populations. These findings provide population-specific toxicological reference data that may assist interpretation of postmortem toxicology results, support identification of emerging substance exposure trends among foreign nationals, and help guide targeted toxicological screening strategies in Thailand.

Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

P-153

Thailand's Poly-Drug Challenge: Kratom Co-Ingestation in Accidental vs Suicidal Fatalities (2019-2024)

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Abstract

Introduction: Following the decriminalization of kratom (Mitragyna speciosa) in Thailand, its presence in forensic autopsies has increased significantly. However, kratom is rarely the sole substance in fatal cases, with poly-drug use occurring in approximately 87% of instances. Understanding the distinct patterns of co-ingestants associated with different modes of death is critical for targeted public health interventions.

Objectives: This study aims to contrast the demographic and toxicological profiles of kratom-positive fatalities classified as "accidental trauma" versus "intentional self-harm".

Methods: A retrospective analysis was conducted on autopsy data from Department of Forensic Medicine, Faculty of Medicine, Chulalongkorn University (2019-2024). A total of 124 cases were included after excluding natural or undetermined deaths. Statistical comparisons were performed using Kruskal-Wallis and Chi-Square tests.

Results: The study cohort comprised 83.9% (n=104) accidental trauma cases and 16.1% (n=20) intentional self-harm cases. A significant age disparity was observed, with intentional self-harm cases being significantly older than those in the accidental trauma cases (Median [IQR]: 39.5 [28.0-51.0] vs. 26.0 [21.0-34.0] years; $p = 0.0066$). While poly-drug use was prevalent in both groups, distinct toxicological patterns emerged. The accidental group demonstrated a significantly higher prevalence of antihistamine co-ingestion (59.6%), strongly indicative of the recreational "4x100" cocktail (kratom, cough syrup, cola). In contrast, among the co-ingested substances analyzed, the self-harm cases exhibited higher rates of alcohol (50.0%) and stimulant (25.0%) co-ingestion.

Discussion: Kratom-associated fatalities are context-dependent events driven by specific co-ingestant combinations and user demographics. Prevention efforts must target the psychomotor impairment from mixing kratom with antihistamines and alcohol to prevent accidents. For the intentional self-harm cases, the co-occurrence of kratom with alcohol and stimulants in self-harm cases emphasis an urgent need for integrated mental health and addiction screening. These distinct profiles necessitate tailored, evidence-based public health strategies in Thailand.

Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

P-154

Fatality Due to Combined Toxicity of Guaifenesin, Methorphan, and Fluoxetine

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Abstract

Introduction: Guaifenesin is the primary ingredient of Mucinex; it also may be combined with other drugs, including dextromethorphan (Mucinex DM). Guaifenesin is an over-the-counter medication used as an expectorant to relieve chest congestion by thinning and loosening mucus in the airways. Dextromethorphan is commonly used as a cough suppressant but can produce feelings of euphoria or dissociative experiences when used recreationally. Both drugs can cause central nervous system depression at high doses.

Here we report the suicidal death of a 33-year-old man from acute combined drug toxicity (methorphan, guaifenesin, and fluoxetine). The decedent was found in a supine position on the bedroom floor in an early state of decomposition. Various prescription medications were observed on scene along with multiple blister packs of Mucinex DM. The decedent had a history of schizophrenia, post-traumatic stress disorder, and Mucinex abuse, for which he had entered rehab in May 2025. Family reported that he was known to take 20-25 Mucinex pills recreationally. Suicidal ideations and attempts were denied by family; however, 'suicide prevention safety plan' paperwork from a medical provider was found on scene.

Objectives: Describe the pathology and toxicology findings associated with this fatality.

Methods: Postmortem specimens were analyzed at the Harris County Institute of Forensic Sciences for volatiles and screened for drugs using an 8-panel ELISA screen, GC/MS, and LC/QTOF MS.

Results: At autopsy, pulmonary and cerebral edema were noted along with urinary retention. The stomach contained 60 mL of green-brown granular liquid. In initial toxicology testing, methorphan (>1.0 mg/L) and norfluoxetine (0.078 mg/L) were identified in heart blood; dextrorphan/levorphanol and guaifenesin were presumptively identified. Benzoylecgonine, carisoprodol, cocaine, fentanyl, methadone, oxycodone, opiates, and other acidic and basic drugs were not detected. Given the decedent's history of abusing Mucinex DM along with the methorphan that exceeded the laboratory's upper limit of quantitation, heart blood and liver were sent to NMS Labs for postmortem expanded panel testing as peripheral blood was not submitted. The results were as follows:

	Heart Blood	Liver
Guaifenesin	140 mg/L	0.10 mg/kg
Dextro / Levo Methorphan	8.6 mg/L	140 mg/kg
Dextrorphan / Levorphanol	0.83 mg/L	5.5 mg/kg
Fluoxetine	ND*	0.30 mg/kg
Norfluoxetine	ND*	4.0 mg/kg

*reporting limit of screen = 0.050 mg/L

Discussion: The cause of death was attributed to the acute combined toxicity of methorphan, guaifenesin, and fluoxetine. The manner of death was classified as suicide. The reported methorphan concentrations are consistent with other reported deaths attributed to methorphan toxicity. The guaifenesin concentration in this case exceeded previously reported levels in cases for which guaifenesin was listed as contributing to death. Based on our review of the literature, the highest guaifenesin concentrations previously reported were 25 mg/L (femoral blood) in a single-drug intoxication and 27.4 mg/L (heart blood) in a mixed-drug intoxication.

Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

P-155

A Study of Deaths Associated with Polysubstance Use at the Central Institute of Forensic Science, Thailand (2022–2025)

Tanasiri Yokchue

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Abstract

Introduction: The illicit drug market has shifted toward complex, highly lethal polysubstance mixtures. Understanding these emerging preparations is critical for advancing toxicological screening and guiding public health interventions. This evolution encompasses the synergistic co-administration of central nervous system (CNS) stimulants with dissociative anesthetics, the integration of atypical chemical adulterants, and the ongoing forensic challenge of differentiating the deliberate diversion of prescription opioids from legitimate medical administration.

Objectives: To investigate the trends and characteristics of polysubstance-related fatalities in Thailand (2022–2025), with a primary focus on illicit narcotics. Specifically, this research highlights the role of ketamine in recreational cocktails and the increasing trend of mixing N,N-Diethyl-meta-toluamide (DEET) with methamphetamine (MA). Furthermore, it aims to clearly distinguish the deliberate abuse of prescription opioids, such as tramadol and fentanyl, from their legitimate therapeutic use.

Methods: A retrospective toxicological analysis was conducted on postmortem biological evidence from 12,462 cases submitted to the Central Institute of Forensic Science (CIFS), Thailand, between 2022 and 2025. Parent compounds were selectively isolated to eliminate analytical duplication from metabolites. Cases involving prescription opioids were rigorously filtered against medicolegal causes of death (e.g., traffic trauma, intensive care hospitalizations) and the co-occurrence of established illicit markers (e.g., MA, mitragynine) to accurately classify recreational misuse versus iatrogenic administration.

Results: Over the four-year study period, toxicological screening yielded 7,260 positive detections. Severe fatal toxicity was heavily driven by synergistic black-market preparations. Ketamine (n=107) functioned predominantly as a co-intoxicant (64.5% of instances), frequently co-administered with MA or MDMA/MDA. Furthermore, the insect repellent DEET emerged as a lethal adulterant in 155 fatalities, predominantly co-detected with MA. Regarding opioids, forensic filtering revealed that 73% of the 550 tramadol-positive cases were therapeutic. However, a distinct subset of 148 cases demonstrated intentional illicit diversion, integrating tramadol into regional depressant cocktails containing mitragynine and first-generation antihistamines. Conversely, of the 145 fentanyl-positive cases, virtually all correlated with severe trauma or ICU treatment, representing palliative hospital care prior to death; no clear pattern of illicit fentanyl misuse was detected.

Discussion: Polydrug fatalities reveal a distinct epidemiological trend in Thailand: rather than adopting Novel Psychoactive Substances (NPS), abusers are increasingly synthesizing highly lethal cocktails by compounding MA with various secondary agents. Within this landscape, ketamine has rapidly emerged as a primary co-intoxicant, alongside the widespread diversion of prescription tramadol into regional depressant formulas. A critical novel finding is the lethal integration of DEET, explicitly co-administered with MA to induce profound dizziness and dangerously potentiate the stimulant's psychoactive effects. Although illicit fentanyl misuse was not detected within this four-

province CIFS dataset—highlighting the need for broader national surveillance—the rapid evolution of these adulterated club drugs demands immediate public health intervention. The unpredictable, synergistic toxicity of mixing CNS stimulants with dissociative anesthetics or chemical adulterants drastically elevates the risk of accidental fatal overdose. Forensic laboratories must continuously adapt screening protocols, and aggressive societal warnings are vital to prevent future fatalities.

Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

P-156

Use of Randox Biochip Array Technology and Correlation to Confirmatory 'Gold Standard' Techniques for Comprehensive Drug Screening

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Abstract

Introduction: Opioid misuse and addiction costs the United States more than $500 billion annually with the CDC's National Center for Health Statistics (NCHS) reporting drug overdose deaths rose >50% in a 6-year period. In 2017, 70,715 reported deaths occurred, increasing to 106,352 in 2023 – with fentanyl, methamphetamine and cocaine classified as the top contributing narcotic substances, highlighting the need for reliable and affordable analysis methods within the forensic science sector, where mass spectrometry is widely regarded as the 'gold standard' for comprehensive drug testing.

Objectives: Randox's Evidence MultiSTAT DOA Urine MultiPlex Array provides a complete screening solution for the simultaneous testing of 15 drugs of abuse (DOA) in human urine.

This study aims to assess array performance and correlation of authentic urine samples which had concentrations confirmed via Mass Spectrometry (LC-MS, LC-MS/MS or GC-MS).

Methods: The MultiPlex Array was applied to the fully automated benchtop analyser Evidence MultiSTAT, which processes a self-contained cartridge comprising all reagents required for sample analysis.

Unadulterated authentic urine samples, obtained from United States based clinical laboratories were assessed on the MultiSTAT Array which produced a qualitative result. Agreement to confirmatory methods was determined.

Results: The Randox Evidence MultiSTAT DOA Urine MultiPlex Array method comparison study was completed using a minimum of 80 authentic, unadulterated human urine samples per analyte, with a variety of drug concentrations, including in the near cut-off range (within +/- 50% of the cut-off). The percentage correlation was determined as follows: Methamphetamine (cut-off: 500 ng/ml) = 96%; Noroxycodone (cut-off: 100 ng/ml) = 80%; Oxazepam (cut-off: 200 ng/ml) = 95%; Methadone (cut-off: 300 ng/ml) = 99%; Phenobarbital (cut-off: 200 ng/ml) = 94%; Tramadol (cut-off 200 ng/ml) = 99%; Phencyclidine (cut-off: 25 ng/ml) = 94%; Buprenorphine (cut-off: 5 ng/ml) = 97%; 6-acetylmorphine (cut-off: 10 ng/ml) = 99%; Fentanyl (cut-off: 1 ng/ml) = 79%; Lorazepam (cut-off: 200 ng/ml) = 95%; Morphine (cut-off: 300 ng/ml) = 92%; Benzoylecgonine/Cocaine (cut-off: 150 ng/ml) = 94%; Cannabinoids (THC) (cut-off: 50 ng/ml) = 91%; Amphetamine (cut-off: 500 ng/ml) = 96%. Discordant samples are accounted for by related compounds that have known cross-reactivity.

Discussion: The Randox Evidence MultiSTAT DOA Urine MultiPlex Array on the Evidence MultiSTAT analyser simultaneously screens for 16 drugs of abuse and has demonstrated a high level of agreement with confirmatory methods, indicating its exceptional applicability for comprehensive drugs of abuse analysis.

Disclosure

Yes, I, or a member of my immediate family, has a financial interest to disclose.

Conflict of Interest

Salary

P-157

Alignment Between DRE-Observed Indicators of Impairment and Toxicology Data in Polysubstance Use Cases

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Abstract

Introduction: Driving impairment due to drugs other than alcohol is difficult to characterize, particularly in polysubstance use contexts. The Drug Recognition Expert (DRE) 12-step protocol is widely used to identify drug impairment through physiological, behavioral, and psychophysical indicators; however, its performance when substances of opposing pharmacodynamic effects are co-used is considered unpredictable. Stimulant-narcotic analgesic combinations (e.g., cocaine and opioids) may produce antagonistic physiological effects that attenuate canonical indicators such as pupil size, pulse, and blood pressure, potentially reducing sensitivity for individual drug categories.

Objectives: To determine which DRE evaluation indicators are masked during polysubstance use, with a specific focus on stimulant-narcotic analgesic co-exposure, by combining officer observations with comprehensive toxicological findings.

Methods: Participants (n=46)* were recruited during DRE field certification evaluations in Milwaukee, Wisconsin (IRB # 2024-1058). Following completion of the standardized 12-step DRE assessment and documentation on Drug Influence Evaluation (FACE) sheets, consenting participants provided self-reported substance use data and biological specimens (urine, whole blood, and oral fluid). Specimens were analyzed using liquid chromatography quadrupole time-of-flight mass spectrometry (LC-QTOF/MS) for broad-spectrum drug and metabolite detection. DRE officer opinions regarding drug categories were compared to toxicological findings and participant self-report, with emphasis on cases involving concurrent stimulant and narcotic analgesic detection.

Results: Polysubstance use was common, with frequent co-detection of stimulants (e.g., cocaine) and opioids (e.g., fentanyl) (co-detection n=25)*; cannabis was also prevalent (n=7)*. In stimulant-narcotic analgesic co-use cases, key physiological indicators, pupil size, pulse, and blood pressure, often fell within average ranges, with large variability, consistent with opposing pharmacological effects. Horizontal and vertical gaze nystagmus were generally absent in these cases. Despite attenuation of individual physiological markers, clues of impairment were often still observed during the psychophysical tests.

Importantly, DRE officers identified both stimulant and narcotic analgesic drug categories in most cases where both were confirmed in biological matrices (blood or oral fluid, n=24)*, although individual indicators for each category were less pronounced. Temporal variability in officer opinions was observed in repeated evaluations conducted within short intervals (45-60 minutes), suggesting dynamic changes in impairment presentation.

Discussion: Stimulant-narcotic analgesic polysubstance use can mask traditional physiological indicators used in DRE evaluations by shifting measures such as pupil size, pulse, and blood pressure towards normative ranges. Despite this masking effect, the overall DRE framework appears robust for detecting impairment and, in most cases, correctly identifying multiple contributing drug classes. These findings highlight the importance of integrating comprehensive toxicological analysis with DRE observations and suggest that reliance on individual physiological markers alone may underestimate impairment in polysubstance contexts.

*As of abstract submission date

Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

P-158

Development of a Portable Capillary Electrophoresis for On-Site Analysis of Illicit Drugs in Oral Fluid

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Abstract

Introduction: Drug-impaired driving represents a serious road safety concern, creating a need for reliable on-site detection tools. Oral fluid is an attractive alternative biological matrix for roadside drug testing, as sample collection is rapid, non-invasive, sex-neutral, and can be directly supervised by law enforcement officers without the need for medical personnel or privacy-compromising procedures. However, current immunoassay-based on-site tests are qualitative and prone to false-positive results, while the reference standard LC–MS requires laboratory infrastructure and trained personnel, limiting its applicability in field settings. Capillary electrophoresis coupled with deep-UV fluorescence detection (CE–FD) enables rapid separation and direct sensitive detection of many drugs of abuse through their native fluorescence, making it attractive for portable drug screening.

Objectives: Building on a proof-of-concept CE–FD device previously developed in our group, this study aimed to develop an improved portable instrument covering a broader analyte panel and fully automated for use by non-specialist personnel, at a cost comparable to immunoassays and capable of providing reliable quantitative identification of multiple analytes on-site within minutes.

Methods: The developed method involves simple, non-invasive oral fluid collection and sample preparation consisting of protein precipitation followed by filtration. Prepared oral fluid samples were analyzed using an automated portable CE–FD device (Drug Hunter, Tallinn University of Technology, Estonia) employing deep-UV excitation and detection of native fluorescence, and compared with a reference LC–MS method. Method applicability was demonstrated by analyzing 22 fortified and 23 authentic samples.

Results: Full testing required 15 minutes per sample and enabled simultaneous quantification of ten drugs commonly associated with driving under the influence of drugs cases, including amphetamine, methamphetamine (LOD 60 ng/mL), MDA, MDMA, MDEA (LOD 0.5 ng/mL), cocaine, cocaethylene (LOD 3 ng/mL), codeine, morphine (LOD 75 ng/mL) and tramadol (LOD 3 ng/mL). Method validation was performed in accordance with the ICH M10 guideline and included specificity, selectivity, within- and between-run accuracy and precision, carryover, matrix effects, extraction recovery, stability and re-injection reproducibility. In both, fortified controls and authentic samples, CE–FD results were confirmed by the reference LC–MS method, except for a single false-positive finding for morphine.

Discussion: A portable, automated CE–FD system for rapid quantitative drug screening in oral fluid was successfully developed. The combination of a simple sampling procedure, automated workflow, and integrated data processing enables on-site drug testing by non-specialist operators. The device is currently deployed for on-site screening by the Estonian traffic police. As it is not feasible to evaluate all compounds potentially present in oral fluid, false-positive results for other analytes cannot be fully excluded. Consequently, individuals with positive screening results are referred for a medical impairment examination and confirmatory analysis at the Institute of Forensic Science.

Disclosure

Yes, I, or a member of my immediate family, has a financial interest to disclose.

Conflict of Interest

Salary - the work was done at Tallinn University of Technology, where the presenting author is a PhD student. Additionally the presenting author is also employee of SafePAS company, where the used device is manufactured.

P-159

Results Following a Two-Year Drug-Impaired Driving Backlog Project for the State of Texas

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Abstract

Introduction: The National Safety Council's Alcohol, Drugs, and Impairment Division (NSC-ADID) publishes recommendations regarding toxicology testing of potentially impaired and deceased drivers; they also emphasize the need for testing to be relevant and up-to-date, which can also mean updated scopes to reflex regional drug trends. Trends in drugs involved in DUID cases have evolved over time, requiring larger scopes and advanced instrumentation, which place significant strain on laboratories. Cross-institutional support from private-sector laboratories can serve as a reliable mechanism for maintaining operational resilience when public-sector forensic systems undergo laboratory modernization efforts, ensuring impaired-driving investigations and prosecutions continue without interruption while gathering timely drug trend information.

Objectives: This study characterizes drug positivity trends observed in DUID cases analyzed during a two-year backlog reduction project, with focus on substances included in the NSC 2021 Tier 1 framework.

Methods: Results from cases analyzed during the two-year DUID backlog project were collated and evaluated for subclass and analyte positivity. Subclass co-positivity will also be evaluated.

Results: Among the 12,785 cases analyzed, more than half tested positive for one or more cannabinoids, with Delta-9 and Delta-9 Carboxy THC being detected in 6,536 and 7,173 blood samples, respectively. Delta-8 THC was found in 262 cases of the same population (3.6% of cannabinoid positive casework). However, Delta-8 THC was found in 576 additional cases (4.5%) in the absence of Delta-9 THC; this data demonstrates the expansion of semi-synthetic cannabinoids in Texas. Stimulants were also commonly found, with approximately 17% of cases testing positive for amphetamine (n=2,192) and/or methamphetamine (n=2,146). Cocaine was only detected in 383 cases (3%); benzoylecgonine (BZE) was found in the absence of cocaine in another 873 cases (9.8%). Benzodiazepines constituted the third most prevalent drug class, with alprazolam as the most frequently reported drug within this category (n=1,449, 11%). Additionally, several designer benzodiazepines were identified, notably bromazolam, which was reported in 353 cases (2.8%). 4'-Chloro deschloroalprazolam was detected in an additional 46 cases (0.36%), as the third most popular designer benzodiazepine in the dataset and a compound fairly regionalized to Texas, after clonazolam (n=41, 0.32%) and its metabolite, 8-aminoclonazolam (n=65, 0.50%). Fentanyl was identified as the most frequently detected opioid, appearing in 449 cases (3.5%); hydrocodone was a distant second at 259 cases (2.0%). Finally, Texas remains among the limited regions with documented and consistent, though comparatively low, PCP positivity; this observation aligns with findings previously reported in the literature, with PCP detected in 252 cases (1.9%).

Discussion: A public-private partnership between laboratories ensured the continuity and scientific integrity of impaired-driving toxicology testing during a period of significant period organizational growth in conjunction with targeted funding from the 2023 Texas legislature. This collaboration effectively avoided the exponential growth of the case backlog and the halting of

impaired driving investigations, which could have led to the jeopardization of public safety efforts. Inter-laboratory cooperation provided a reliable approach for sustaining operational resilience during public-sector laboratory modernization efforts, including the purchase and validation of new instrumentation, while capturing trend data that is largely expected (i.e. cannabinoids) as well as emerging patterns.

Disclosure

Yes, I, or a member of my immediate family, has a financial interest to disclose.

Conflict of Interest

Grant/Research Support and Salary/Consultant

P-160

Characteristics of Alcohol- and Drug-Impaired Driving Reoffenders in Houston, 2019-2025

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Abstract

Introduction: Driving under the influence of alcohol or drugs is an offense for which some individuals are repeatedly arrested. Many explanations for why a driver may reoffend have been proposed (e.g., risk-taking behavior, substance abuse, ineffective sanctions). Identifying recidivism patterns among offenders could provide insight to support law enforcement and public health outreach efforts.

Objectives: This study's aim was to determine the extent to which the laboratory receives reoffenders' blood samples, and to identify characteristics of these individuals compared to drivers with single offenses.

Methods: The laboratory performs alcohol analysis on all cases using headspace gas chromatography (GC)-flame ionization detection; fatalities and/or alcohol <0.10 g/100 mL are screened via immunoassay. Fatality cases receive an expanded GC-mass spectrometry (MS) basic drug screen. Positive results are then confirmed either by GC-MS or liquid chromatography-MS/MS. Driving offenses between 1/1/2019 and 12/31/2025 were identified. Individuals with dates of birth and names that appeared at least twice were identified as repeat offenders and further separated by alcohol positivity.

Results: Cases tested with offenses occurring 2019-2025 numbered 25,455. Of these, 2,818 cases were associated with 1,297 reoffending drivers. After 151 reoffenders were removed for having mixed alcohol and drug detection in their cases, 1,146 recidivists remained. Table 1 lists characteristics of the drivers.

	Alcohol-Positive	*Alcohol-Negative*					
Offenses per driver	**N**	**Mean BAC, g/100 mL**	**Mean (median) age at first offense, years**	**Sex, (%M)**	**N**	**Mean (median) age at first offense, years**	**Sex (%M)**
One	18497	0.174	36.7 (34.3)	72	4333	36.6 (35.3)	71
Two to five	770	0.185	35.2 (32.5)	80	376	37.5 (37.9)	74

Table 1. M=male, W=White, B=Black.

Mean BACs for reoffenders were higher than for single offenses; drivers with two and three offenses had a mean BAC of 0.184 and 0.195 g/100 mL, respectively. One driver had a mean BAC of 0.125 g/100 mL across five alcohol-positive offenses. Two alcohol-negative drivers were positive for drugs in each of their five offenses (benzodiazepines/cannabinoids/phencyclidine for one, and opioids/carisoprodol for the other). Alcohol-positive reoffenders had a mean age 1.5 years younger at their first offense than single offenders; conversely, alcohol-negative reoffenders were 0.9 years older at first offense than single offenders. Race data had limited value due to inconsistency in how Hispanic origin was entered in the case submission information and was not examined further.

Discussion: Reoffenders accounted for 11% of casework between 2019-2025. This is markedly lower than in Finland, where one-third of all arrested drivers were re-arrested in a 15-year period (Impinen et al. 2009). The shorter, six-year observation window here likely underestimates the true rate of reoffending in Houston.

Males far exceeded females in samples tested, though they are 49% of the Houston population. Alcohol-positive reoffenders were younger at first offense than single offenders; the reverse was true for alcohol-negative reoffenders. Along with the higher BACs observed for reoffenders, these data highlight the need to aim substance use intervention efforts at young, especially male, Houston drivers.

Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

P-161

How Long It Takes to Obtain a DUI Blood Draw in Wyoming and Other DUI Statistics From the Cowboy State

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Abstract

Introduction: Wyoming is the least populated state in the U.S., with only about 588,000 residents. It is the second most rural state in America with a population density of about 5.9 people per square mile. The distance between major towns can often be 50-150 miles. This can be a challenge in DUI investigations, when transportation to the nearest blood draw facility is lengthy. Wyoming routinely ranks amongst the worst states for levels of DUI. In 2024, over 57% of individuals arrested for DUI had a BAC of 0.16 or higher and 21% of individuals had a BAC of 0.24 or greater.

Objectives: This presentation aims to provide information about the types of drugs that are most commonly found in Wyoming DUI investigations in combination with alcohol. BAC concentrations obtained from Wyoming DUI drivers will be presented and the average time it takes to obtain a blood draw will also be shared.

Methods: All toxicology samples are analyzed for alcohol and/or drugs based on the request from the submitting agency. Alcohol and other volatiles testing is performed using headspace gas chromatography with a flame ionization detector (HS/GC-FID). A 15 panel ELISA drug screen is followed by drug confirmation utilizing a liquid-liquid extraction and instrumental analysis via tandem mass spectrometry (LC-MS/MS). Drug confirmation can currently be performed for the cannabinoid, basic drugs, and benzodiazepine drug classes. Data presented is from the 2025 calendar year.

Results: The Wyoming State Crime Lab analyzed 2,147 cases in 2025. The average time it took to obtain a blood draw after the incident was 1 hour and 58 minutes. The time elapsed between the incident and the draw ranged from 34 minutes to more than 5 hours. The time between incident and draw was consistent between agency types, such as police departments, sheriff's offices, and the Wyoming Highway Patrol. Following alcohol analysis, 76% of the cases had a BAC greater than 0.08 and 56% of the cases had a BAC greater than or equal to 0.16. The most common drug detected with alcohol is THC, with 42% of cases that are positive for alcohol also being positive for THC or THC metabolites. Other popular polydrug combinations are cannabinoids + methamphetamine (13%), cannabinoids + methamphetamine + alcohol (8%), and cannabinoids + benzoylecgonine + alcohol (5%).

Discussion: The amount time time it takes to obtain a blood draw in Wyoming is similar to the state of Colorado, where it also takes about two hours. However, it is unknown if the time gap is similar to states with a comparable population density. Alcohol is the most common substance detected in Wyoming DUI drivers with THC being the second most common drug detected. Stimulants are also seen in combination with alcohol and THC, which is consistent with neighboring states.

Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

P-162

Assessment of Tissue Homogenization Methods for NPS Benzodiazepine and Fentanyl Analogues

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Abstract

Introduction: In recent years, the increasing prevalence of novel psychoactive substances (NPS) within opioid and benzodiazepine classes has added complexity to forensic toxicology casework, creating challenges for both public health and analytical detection and interpretation. Traditional matrices include blood and urine, with vitreous humor commonly used in postmortem investigations. However, these may not always be available and are often used in combination to support interpretation. Postmortem redistribution further complicates the use of blood concentrations, highlighting the value of alternative matrices, such as tissues, to better characterize analyte distribution. Despite this potential, limited research exists on drug concentrations in tissues, particularly for novel drugs. Consequently, blood-tissue concentration relationships for emerging drugs remain poorly understood. To address this, laboratories require effective methods to extract analytes from tissue for accurate detection and quantification.

Objectives: This study aimed to optimize recovery of selected NPS opioids and benzodiazepines in tissue matrices by evaluating homogenization methods to maximize recovery, minimize variability, and improve analytical reliability.

Methods: The analytes in this study were bromazolam, clonazolam, desalkylgidazepam, etizolam, fentanyl, flualprazolam, flubromazepam, flubromazolam, *ortho*-methylfentanyl, and *para*-fluorofentanyl. Tissue matrices included bovine and poultry heart and liver. Using a previously validated solid phase extraction for blood, four different tissue homogenization methods (4X dilution with water, 10 sources) were explored to optimize analyte recovery: (1) handheld probe; (2) bead mill, 1.4 mm ceramic beads; (3) bead mill, 1.4 mm ceramic beads and post-extraction filtration; and (4) bead mill, 2.8 mm ceramic beads (water or methanol diluent). Homogenates (250 µL) were prepared as pre- and post-fortified extracts for each homogenization type. Analysis was performed on an Agilent 1290 Infinity II LC with an Agilent 6470 LC-MS/MS using positive electrospray ionization and multiple reaction monitoring. Performance of each method was determined by calculating analyte recovery and reproducibility (precision, %CV).

Results: Recoveries and precision varied across techniques methods. Method 1 produced recoveries of 30.8-70.4% (6.1-51.0 %CV), method 2 improved recoveries to 51.3-80.1% (2.7-38.1 %CV), and method 3 showed broader variability (22.0-72.0% recovery, 12.9-51.9 %CV). Method 4 provided more consistent performance with both diluents, producing recoveries of 44.8-70.9%, %CVs of 4.2-23.0%, and had the greatest overall consistency in visual matrix homogeneity.

Discussion: The optimal homogenization method used a bead mill with 2.8 mm ceramic beads and a 4X dilution with water in both matrices. Although some analytes did not meet 50% recovery or %CV acceptance criteria (<20%), this approach demonstrated the greatest overall reproducibility. Initially, method 4 utilized methanol for liver samples and water for heart samples due to differences in homogenate viscosity; however, when assessing only heart matrices, all recoveries and %CVs were acceptable. Therefore, it was determined that improvements

in recoveries from the other methods were due to the increase in bead size rather than the homogenization solvent used. Additionally, matrix effects were within ±25% using the fourth technique. Overall, these results provide a practical foundation for laboratories seeking to incorporate tissues into their extractions workflows, offering guidance on method development and highlighting key considerations for achieving reliable and reproducible results.

Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

P-163

Withdrawn

Withdrawn

P-164

Formalin-Fixed Paraffin-Embedded Tissue: A Suitable Matrix for Drug Screening?

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Abstract

Introduction: Formalin-fixed paraffin-embedded (FFPE) tissue is used for histological diagnostics. Due to its long-term storage capability, it is interesting for retrospective toxicological analyses. However, its preparation process causes drug elution from tissue in formalin solution and organic solvents, reducing tissue concentration significantly. Additionally, formaldehyde can react with drugs in many different ways, forming conversion products that are not methodologically implemented. Of those reactions, Eschweiler-Clarke conversion is the most common, leading to N-methyl and N,N-dimethyl analogues.

Objectives: To evaluate the detectability of drugs in FFPE-tissue, a new qualitative drug screening method has been developed and validated.

Methods: FFPE-lung tissue was simultaneously homogenized and extracted in acetonitrile including a salting-out assisted liquid-liquid extraction and a filtration step as clean-up. Analysis was carried out with LC-QTOF high resolution mass spectrometry in data independent acquisition mode. The method was validated by evaluating the parameters selectivity, limit of detection, recovery and matrix effects, and stability for 19 analytes distributed throughout the chromatogram, and applied to 10 post-mortem cases with previously performed toxicological analysis in femoral or heart blood.

Results: Validation was achieved according to the guidelines of the Society of Toxicological and Forensic Chemistry (GTFCh).

Drug findings in real samples include psychoactive substances and medications like amphetamines, anticonvulsants, antidepressants, antipsychotics, benzodiazepines, and opioids.

Additionally, the chromatograms were analysed considering derivatives formed by plausible drug-formaldehyde reaction mechanisms. Thus, N,N-dimethylamphetamine (DMA) was detected, and furthermore, indications of 3,4-Methylenedioxy-N,N-dimethylamphetamine (MDDMA) were obtained.

Discussion: Drug findings in FFPE tissue showed generally good consistency with prior blood analyses. However, low drug concentrations and partial conversion after formalin exposure leads to risks of false negative results, which might be relevant in cases of single therapeutic dose administration shortly before death. For example, in one case the emergency medication ketamine was not observed, whereas cafedrine, midazolam, the antidepressant citalopram, and the anticonvulsants carbamazepine and levetiracetam were detected. Therefore, the case history can provide important information to obtain and interpret analytical data.

The detection of DMA in FFPE-lung tissue in one case shows that reactions between drugs and formaldehyde had occurred. An equivalent conversion was observed for 3,5-methylenedioxy-methamphetamine (MDMA) to MDDMA as well.

Drug screening in lung FFPE-tissue appears to be a useful approach in acquiring toxicological data, when other specimens are no longer available. Drug findings in blood and FFPE-lung tissue blocks correlate well. However, quantitative interpretation seems to be difficult because of influences referring to initial drug administration, case history, and FFPE tissue preparation. For a final evaluation, further research regarding this novel matrix is needed.

Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

P-165

The Bones Never Rest: Determination of Alkaloids in Bones in an Archeo-Toxicological Context

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Abstract

Introduction: Archaeo-toxicology is an emerging scientific field that explores archaeological materials through toxicological analysis. Most research has focused on skeletonized remains to study heavy metal exposure, but few studies have looked into organic substances like alkaloids in bones.

Objectives: The goal of our study was to develop and validate an analytical method for the determination of morphine, codeine, thebaine, noscapine, papaverine, ephedrine, atropine, and scopolamine in bones using liquid chromatography-tandem mass spectrometry (LC-MS/MS).

Methods: Cleaned bones (chicken and rib bones) were ground in a mortar and pestle, then 200 mg of small bone pieces were incubated in 3 mL of methanol in a sonicator at 50°C for 1 hour, followed by incubation at room temperature on the benchtop for 72 h in the presence of the internal standard (50 µL of 0.1 µg/mL). After incubation, the tubes were centrifuged, and 2.5 mL of supernatant was collected. Before evaporation, 50 µL of 1% HCl in methanol was added to prevent ephedrine loss. The samples were reconstituted with 200 µL of MPA (0.1% formic acid in water), vortexed for about 30 s, and centrifuged for 10 min at 5000 rpm. Approximately 175 µL of supernatant was collected and transferred to 0.2 µm nano filter vials before being analyzed by LC-MS/MS. Chromatographic separation was performed on a reversed-phase Kinetex C18, 2.6 µm particle size, 2.1 x 100 mm, chromatographic column from Phenomenex (Torrance, CA) with 0.1% formic acid in water and in acetonitrile as the mobile phase. The LC-MS/MS was operated in positive electrospray ionization mode (ESI+), and two MRM transitions were monitored per compound. The method was validated following the ANSI/ASB 036 standards.

Results: Linearity ranged from 1 to 100 ng/g, with acceptable bias of -12.8 to 5.0% (n=12) and imprecision <19.8% (n=12), evaluated at low QC (3 ng/g), medium QC (30 ng/g), and high QC (75 ng/g). Regarding matrix effects (n=8), all alkaloids, except thebaine, showed ion suppression (-52% to -66%), while thebaine showed ion enhancement (56%) at medium QC. For extraction efficiency (n=8), all compounds had extraction efficiency of at least 50% at medium QC. No carryover at 100 ng/g or endogenous interference from 10 bones were observed. Morphine failed validation due to inconsistent chromatography and peak intensity.

Discussion: A sensitive and specific method was created for the determination of seven alkaloids in 200 mg of bone, including codeine, thebaine, noscapine, papaverine, ephedrine, atropine, and scopolamine. This procedure will be applied to authentic human bones from an archeological site in Menorca, Spain, dating to 1,000 BCE, where the use of *Ephedra, Atropa belladonna*, and *Papaver somniferum* was documented. This method will have to be adjusted to include morphine before it can be applied to the authentic bones.

Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

P-166

LC-MS/MS Detection of Cannabinoid Metabolites in Umbilical Cord Tissue for Assessment of Prenatal Cannabis Exposure

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Abstract

Introduction: Prenatal cannabis exposure is increasing with expanding legalization and increased cannabis access. Cannabis contains multiple biologically active cannabinoids, including Δ9-tetrahydrocannabinol (Δ9-THC) and cannabidiol (CBD), as well as emerging isomers such as Δ8-THC. Neonatal drug testing traditionally focuses on Δ9-THC-COOH, which may underestimate exposure to diverse cannabis products and metabolites. Additional markers, including Δ8-THC-COOH and 7-COOH-CBD, may improve detection of exposures to emerging cannabinoid products and broaden analytical coverage beyond traditional testing. Umbilical cord tissue (UCT) is a non-invasive matrix reflecting cumulative third-trimester exposure; however, cannabinoid metabolites are frequently present as glucuronide conjugates. The impact of different hydrolysis strategies on cannabinoid recovery and detection has not been fully characterized.

Objectives: To develop and validate an LC-MS/MS method for simultaneous detection of Δ9-THC-COOH, Δ8-THC-COOH, and 7-COOH-CBD in UCT and evaluate the impact of hydrolysis on analytical performance and detection sensitivity.

Methods: De-identified UCT specimens (n=252) submitted for clinical testing were utilized. Tissue samples were homogenized using a bead-mill homogenizer in methanol containing isotopically labeled internal standards. After centrifugation, supernatants were evaluated using both alkaline and enzymatic hydrolysis approaches, with β-glucuronidase selected for final method validation followed by solid-phase extraction. Chromatographic separation was achieved using a Polar C18 column with an isocratic hold at 70% methanol with formic acid. Detection was performed on a SCIEX 6500+ mass spectrometer. Method validation followed CLSI/ ASB guidelines. Due to limited availability of verified negative UCT, urine-based calibration was used (2.5-50 ng/mL) and matrix effect equivalency was evaluated through spike-recovery studies in authentic negative UCT specimens. The reporting cutoff of UCT was 5 ng/g.

Results: Initial alkaline hydrolysis resulted in low or undetectable signals for both THC-COOH and 7-COOH-CBD in UCT specimens, suggesting matrix-dependent analyte loss or degradation. In contrast, enzymatic hydrolysis yielded consistent and reproducible performance, with hydrolysis efficiency of 92.63% at 2000 ng/mL and reduced susceptibility to matrix effects. The validated method showed excellent linearity ($r^2 > 0.99$), accuracy (97–101%), and intra- and inter-day precision (%CV: 1.88–7.04%). No analytical interferences or carryover was observed in this assay. Spike-recovery studies in 15 authentic negative UCT demonstrated equivalency of urine-based calibration within the UCT matrix, with %CVs of 3.3-5.3% at 2.5 and 40 ng/mL. Analysis of 252 clinical UCT remnant specimens identified 16 positives for Δ9-THC-COOH (ranging 5.2 ng/g-81.9 ng/g) and 2 positives for Δ8-THC-COOH (7.3 ng/g and 47.0 ng/g); no specimens were positive for 7-COOH-CBD. Importantly, enzymatic hydrolysis resulted in 13 additional reportable positive specimens that would otherwise have remained below the 5 ng/g reporting cutoff, highlighting the substantial impact of hydrolysis on clinical detection sensitivity. Measured concentrations in positive specimens were approximately threefold higher following enzymatic hydrolysis.

Discussion: Hydrolysis is a critical determinant of cannabinoid detection in UCT, with enzymatic hydrolysis substantially improving analyte recovery and detection sensitivity compared with alkaline approaches. The limitations of alkaline hydrolysis highlight the importance of matrix-specific method optimization for UCT testing. Inclusion of Δ8-THC-COOH broadens analytical coverage beyond traditional Δ9-THC-COOH testing and may improve identification of exposures to emerging cannabinoid products. Overall, enzymatic hydrolysis improved analytical sensitivity and substantially increased prenatal cannabis exposure detection in UCT.

Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

P-167

Stability of Methamphetamine Concentrations in Vitreous Humor Across Prolonged Postmortem Intervals

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Abstract

Introduction: In forensic toxicology, interpreting postmortem drug concentrations is often complicated by the phenomenon of postmortem redistribution and degradation, where drug levels in heart blood (HB) can shift significantly after death. While femoral venous blood (FV) remains the preferred specimen for quantitation, its availability is not always guaranteed. Vitreous humor (VH), protected within the ocular globe, offers a physically isolated environment that is relatively resistant to the rapid putrefactive changes and external contamination that affect blood.

Objectives: In this study, we determined methamphetamine (MA) concentrations in VH, FV, HB, and urine (UR) from forensic autopsy cases positive for amphetamines using LC-MS/MS and evaluated their correlation taking postmortem interval into account.

Methods: The samples of VH, FV, HB and UR were collected from 24 forensic autopsy cases. The postmortem intervals ranged from 1.5 to 10.5 days. Twenty-three cases with a confirmed cause of death (COD) were classified into three groups: group A, acute MA intoxication (n = 7); group B, mixed causes including MA intoxication (n = 8); and group C, non-MA related deaths (n = 8). 0.05 mL of each sample was mixed with 0.05 mL of ultrapure water and 0.4 mL of acetonitrile containing d5-diazepam as an internal standard, vortex-mixed for 1 min, and centrifuged at 12,000 g for 5 min. The supernatant was diluted fourfold with ultrapure water. 10 µL of the diluted solution was analyzed by LC-MS/MS (Triple Quad 5500+, SCIEX). Statistical analyses were performed using JMP® Student Edition 19.0.5. Correlations were evaluated using linear regression analysis, Pearson's correlation coefficients and receiver operating characteristic (ROC) curve. Group differences were assessed using Student's t-test. A p value of < 0.05 was considered statistically significant.

Results: The linear regression equations of MA concentrations were VH (y) = 1.90 FV (x) + 0.249 (r = 0.94), HB (y) = 1.49 FV (x) + 0.248 (r = 0.91), and UR (y) = 40.9 FV(x) – 1.07 (r = 0.64). Better correlation of MA concentrations between UR and FV was obtained from 22 cases with MA concentrations in UR < 40 µg/mL (r = 0.86). MA concentrations in VH ranged from 0.21 to 3.0 µg/mL in group A, from 0.37 to 4.43 µg/mL in group B, and from 0.08 to 0.86 µg/mL in group C. Significant differences in MA concentrations were observed between group A and C, and B and C. Based on ROC curve analysis (AUC = 0.93), MA intoxication could be indicated when MA concentration in VH is > 0.86 µg/mL. Correlation coefficients between postmortem interval and VH/FV, HB/FV and UR/FV ratios of MA concentrations were 0.05, 0.05 and 0.19, respectively, indicating no significant correlation.

Discussion: MA concentrations in VH showed the strongest correlation with those in FV among the specimens regardless of postmortem interval. MA concentrations in VH could be useful for assisting in the determination of the COD.

Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

P-168

Identifying Glucuronide Substrate Biases in β-glucuronidases for Toxicology Applications

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Abstract

Introduction: Opiate and opioid metabolism primarily occurs in the liver, and these drugs are removed from the body by glucuronidation and sulfation to facilitate excretion in urine. Drug testing laboratories can quantify free opiates and opioids in urine by LC-MS/MS after hydrolysis with a β-glucuronidase (β-GUS) and/or sulfatase. We have shown in previous studies that glucuronidated substrates and glucuronidases have different pH preferences for optimal hydrolysis. Use of a single β-glucuronidase can constrain the recovery of glucuronide conjugates, potentially leading to incomplete detection of drug metabolites. Here, we show that individual enzymes exhibit substrate biases, and that combining multiple β-glucuronidases enhances hydrolysis efficiency across a diverse panel of glucuronidated compounds.

Objectives: Compare the activities, or hydrolysis efficiencies, of commercially available β-glucuronidase enzymes on various glucuronidated substrates by testing a range of enzyme concentrations at fixed pH and substrate amounts.

Methods: Deuterated, glucuronidated and parent reference standards were purchased from Cerilliant, Cayman Chemical or Toronto Research Company. All other reagents were purchased from MilliporeSigma or Fisher Scientific. Three β-glucuronidases were expressed and purified at IMCS: *Aspergillus* chimera β-GUS (AspGUS), *Eubacterium eligens* β-GUS (EelGUS), and *Brachyspira pilosicoli* β-GUS variant (BpiGUS). Samples were buffered with sodium acetate at pH 5.5. Reactions were stopped by adding methanol and filtering through a Phenomenex β-Gone plus plate. Samples were further diluted and injected on a Thermo Scientific™ Vanquish™ UHPLC system coupled with a Thermo Scientific™ Endura™ Triple Quadrupole Mass Spectrometer. Analytes were separated using a Phenomenex Kinetex® 2.6 µm Biphenyl 100 Å, 50 x 4.6 mm column. Mobile phase A and B were 0.1% formic acid in water and 0.1% formic acid in acetonitrile, respectively.

Results: BpiGUS variant performed best on glucuronidated codeine, O-desmethyl-venlafaxine, trifluoperazine and haloperidol. Chimeric enzyme abbreviated as AspGUS performed best on glucuronidated morphine and oxymorphone. EelGUS performed best on glucuronidated O-desmethyl-tramadol, imipramine, amitriptyline and cotinine. Activities of each enzyme on the glucuronidated metabolites are summarized in Table 1.

Table 1. Summary of Beta-Glucuronidase Activities (mU/mg).

	BpiGUS(mU/mg)	AspGUS(mU/mg)	EelGUS(mU/mg)
Cotinine	1.3	5.0	5.1
Morphine	2.4	9.0	7.0
Oxymorphone	2.5	7.4	1.6
Codeine	9.2	0.1	7.4
O-desmethyl-tramadol	3.1	3.7	6.1
O-desmethyl-venlafaxine	1.6	1.2	0.2
Haloperidol	18.1	0.0	0.1
Imipramine	1.5	0.3	3.4
Amitriptyline	4.3	0.0	6.5
Trifluoperazine	63.2	1.1	28.8

Discussion: Individual β-glucuronidases exhibit substrate bias. By combining enzymes, the mixture broadens substrate coverage and reduces the risk of incomplete hydrolysis. The highest working enzyme concentration (0.045 mg/mL) comprised 5% of the total reaction volume and was removed during β-Gone plus clean up. Matrix effects from the enzyme were found to be statistically insignificant.

Disclosure

Yes, I, or a member of my immediate family, has a financial interest to disclose.

Conflict of Interest

Authors are employees of Integrated Micro-Chromatography Systems

P-169

Withdrawn

Withdrawn

P-170

The Analysis of Microbial Contamination in Electronic Cigarette Liquids and Generated Aerosols

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Abstract

Introduction: Despite ongoing efforts by the FDA to regulate electronic cigarettes (e-cigs), thousands of unregulated products associated with significant quality assurance gaps remain in the market. Microbial toxins detected in e-cig liquids (e-liquid) have been linked to unsanitary manufacturing practices, improper storage conditions, and contaminated raw materials, including the tobacco and cannabis source materials. The United States Pharmacopeia (USP) has established thresholds for acceptable microbiological contamination in nonsterile formulations based on the route of administration. Microbial levels exceeding these limits may be associated with adverse health effects: aerobic count (AC) > 100 colony-forming units (CFU)/mL or g, yeast and mold > 10 CFU/mL or g, and *Escherichia coli* (*E. coli*)/coliform > 0 CFU/mL or g. While chemical contaminants like vitamin E acetate are recognized as the primary cause of e-cigarette or vaping use-associated lung injury (EVALI), inhaled microbes may increase lung inflammation and compromise respiratory defenses further, potentially worsening the severity of EVALI in affected individuals.

Objectives: This study analyzed microbial contamination in e-liquids and aerosols from confiscated and commercially purchased e-cigs, and evaluated whether specific e-liquid components support bacterial propagation.

Methods: Vaping products confiscated from minors during the 2024–2025 school year were categorized by Virginia's five health districts, product composition (nicotine- or cannabinoid-based), and mouthpiece condition (visibly clean or dirty). E-liquids and aerosols collected using an in-house trapping system, from confiscated and purchased devices, were tested for yeast, mold, AC, *E. coli*, and coliform. Samples were considered positive based on USP thresholds above. Bacterial propagation was assessed using a Cerillo Stratus Kinetic Microplate Reader, where *Bacillus cereus* T-strain was spiked into individual e-liquid matrices and optical density (OD) was measured over time.

Results: No microbial growth was detected in purchased products (n=7). Microbial contamination was detected in ~30% of confiscated e-liquids and aerosols (total n=133), including both nicotine and cannabinoid-containing devices, with half exceeding USP thresholds. In e-liquids, CFU/mL ranged from 0–20 for yeast, 0–50,000 for mold, 0–400,000 for AC, and 0–1,100 for coliform. In aerosol samples, CFU/g ranged from 0–22 for yeast, 0–19 for mold, and 0–2,288 for AC. Of the three products (1 nicotine, 2 cannabinoid) that showed microbial growth in both e-liquids and aerosols, one cannabinoid product was positive—above USP thresholds—in both matrices: mold [13 CFU/mL] and AC [252 CFU/g]. Additionally, cannabidiol (CBD) showed modest bacterial propagation (average $\Delta OD=0.086$).

Discussion: While no purchased products contained microbes, viable microbes were identified in confiscated nicotine- and cannabinoid-containing e-liquids and aerosols, demonstrating that e-cig users may inhale microbes at levels exceeding regulatory thresholds. Low e-liquid microbial counts and limited propagation suggest contamination likely originates from improper storage and unsanitary handling. However, samples exceeding USP thresholds and evidence that CBD-containing products may favor microbial growth raise public health concerns, as repeated use could increase microbial exposure. Such contamination may contribute to fever, rash, gastrointestinal illness, respiratory disease, and potentially severe outcomes, while also complicating the recognition of e-cig-related health effects, including EVALI. These findings highlight the need for improved regulation and public education.

Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

Conflict of Interest

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P-171

Clinical Characteristics and Identified Drugs of Abuse Among People who Use Drugs Admitted to a Tertiary Hospital in the Philippines Using a Validated LC-QTOF/MS Method

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Abstract

Introduction: Drug use and abuse is a public health issue that has come into focus in the Philippines in the recent years. Excluding the years of the COVID-19 pandemic, there has been a yearly increase in the number of admissions to treatment and rehabilitation centers. The census in the University of the Philippines-Philippine General Hospital (UP-PGH) National Poison Management and Control Center (NPMCC) shows a parallel increase in drug-positive patients consulting in the emergency room (ER).

Objectives: The objective of this study was to describe the demographic, clinical characteristics, and drug use profiles of substance users admitted to the UP-PGH and referred to the NPMCC for drug testing.

Methods: This is a cross-sectional study where participants included patients aged 10 years and above who were referred to the NPMCC for drug testing within three days of the ER consult. Once consent, or assent from children, was obtained, patients were interviewed and examined. Urine samples were collected for drug screening using drugs of abuse screening test kits. A split sample was sent to the UP Drugs of Abuse Research Laboratory (UP DARL) for analysis using the liquid chromatography quadrupole time-of-flight mass spectrometry (LC-QTOF/MS). The results were analyzed and summarized using descriptive statistics.

Results: Three hundred eighty-four (384) individuals participated in the study and submitted urine samples for testing from January 2019 to February 2020. One hundred thirty-four (134) samples were positive for substances of abuse detected by drug screening test kits for methamphetamine (MAP), delta-9-tetrahydrocannabinol (THC), cocaine, 3,4-methylenedioxymethamphetamine (MDMA), benzodiazepines, and opioids, and by LC-QTOF/ MS analysis. Majority of the patients (75.37%) were males with an average age of 34.54 ± 1.16 years old. Many complained of neurobehavioral changes necessitating consultation at the hospital emergency room. The

neurologic and cardiovascular systems were frequently affected. By using the drugs of abuse test kit, methamphetamine was the most common substance of abuse detected and was seen in 40.3% of the samples. Amphetamine type stimulants were the most common group of drugs identified by LC-QTOF/MS analysis and were seen in 103 instances. New psychoactive substances detected most frequently than others include paramethoxymethamphetamine (PMMA), 3,4- methylenedioxy methamphetamine (MDMA) and 3,4-methylenedioexyamphetmaine (MDA). A few cathinones like butylone and cathinone were also detected.

Discussion: Methamphetamine was the most common substance of abuse detected in urine samples of the participants. New psychoactive substances were also detected in urine samples when LC-QTOF/MS analysis was utilized. Most persons who use drugs are unemployed young- to mid-adult males. The participants often had neurobehavioural and cardiovascular signs and symptoms.

Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

P-172

Quantification of 2,5-Hexanedione in Urine Using Liquid Chromatography Coupled with Tandem Mass Spectrometry

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Abstract

Introduction: Chronic exposure to n-hexane remains an important occupational health concern because of its well-recognized neurotoxicity. Urinary 2,5-hexanedione (2,5-HD), the major neurotoxic metabolite of n-hexane, is widely regarded as a key biomarker for biological exposure assessment. While gas chromatography-based methods have traditionally been employed for 2,5-HD analysis, they often exhibit a poorer limit of detection (LOD) and are less adaptable to multiplexed or extended biomarker analysis. In contrast, liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS) provides a more flexible analytical platform that is better suited for streamlined workflows and potential expansion toward broader toxicological applications.

Objectives: The objective of this study is to develop and validate a rapid and reliable LC-MS/MS method for the determination of 2,5-HD in human urine.

Methods: Analysis was conducted on a SCIEX ExionLC system coupled with a SCIEX QTRAP 6500+ mass spectrometer. Separation was achieved on a biphenyl column using a 7-minute gradient program, and detection was performed in multiple reaction monitoring (MRM) mode. Method validation was conducted in accordance with ICH M10 guidelines, including assessment of linearity, sensitivity, accuracy, precision, carryover, and matrix effect. Extraction recovery was also evaluated as an additional performance parameter.

Results: The method achieved an LOD of 100 ng/mL and an LOQ of 200 ng/mL. Calibration was linear over 200–2000 ng/mL with $R^2 > 0.990$. Both intra-day and inter-day accuracy and precision met predefined acceptance criteria (within ±15%). Minimal carryover, acceptable matrix effect (with both accuracy and coefficient of variation (CV) within ±15%), and consistent extraction recovery (within 80-120%) further demonstrated the robustness of the assay in urine samples. The use of LC-MS/MS with MRM detection provided high analytical selectivity and minimized potential interferences in complex biological matrices. In addition, the LC-MS/MS platform enables future integration of structurally related metabolites or toxicity-associated biomarkers, which is less readily achievable with conventional GC-based approaches.

Discussion: A rapid and validated LC-MS/MS method for urinary 2,5-HD was successfully established. The method is suitable for routine occupational biomonitoring and offers a practical and scalable analytical platform for future exposure- and toxicity-related biomarker studies.

Disclosure

No, I, nor any member of my immediate family, has a financial interest to discl

P-173

Withdrawn

Withdrawn

P-174

Assessing the Prevalence of p-Phenylenediamine and Heavy Metals in Henna Tattoo Inks Purchased in Northern Delaware

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Abstract

Introduction: The growing popularity of henna tattoos in the United States has raised concerns for public health regarding ink safety [1]. Para-phenylenediamine (PPD) is a potent allergen and carcinogen which is added into henna to extend tattoo lifespan. PPD has been banned by the FDA for direct skin application [2]. However, due to administrative loopholes, cosmetic and professional products are the exception to The Fair Packaging and Labeling Act (FPLA) which regulates mandatory English ingredient labeling. Furthermore, because henna is plant-derived, the phytoaccumulation of heavy metals such as lead, cadmium, and arsenic raises a secondary concern for systemic toxicity [3].

Objectives: This study determines the prevalence of PPD and heavy metals in henna ink purchased from Asian grocery stores in Northern Delaware, assessing variance across ink colors and manufacturers.

Methods: Fourteen henna tattoo ink samples and two hair dyes (positive controls) were assessed. PPD analysis was reduced to an under 4-minute runtime using a modified Wang and Krynitsky [4] method optimized for a DB5-MS column. The heavy metals' quantification was conducted via ICP-MS, following microwave assisted digestion of 0.25 grams of each sample with 0.2 mL HNO3 and 0.8 mL H2O2 diluted to a final volume of 25 mL prior to analysis.

Results: The method detection limit (MDL) and the limit of quantitation (LOQ) were 8ppb and 40ppb, respectively. PPD was confirmed in the positive control (24,000ppm) as expected and identified in a black henna ink sample at 1 ppm. As arsenic and cadmium remained below 1 ppb and lower, lead concentrations were more variable, with several samples containing concerning levels between 1-10ppm.

Discussion: Despite current legislation, the potential for PPD adulteration and variable toxic lead concentrations exists in commercially available henna inks. Future steps are to expand sample size and to develop a DART-MS PPD method to simplify sample preparation and throughput.

Disclosure

Yes, I, or a member of my immediate family, has a financial interest to disclose.

Conflict of Interest

Registration and travel paid for by NSF Award 2306577.

P-175

Surveillance of Novel and Multi-Chamber Vaping Devices

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Abstract

Introduction: Continued innovation within the vaping market has led to the emergence of novel product designs, including multi-chamber systems capable of delivering nicotine and cannabinoids. These multifunctional devices can contain multiple formulations with varying active ingredients, flavors, and cooling agents. They often feature increased e-liquid capacity and incorporate "fidget" or entertainment components. These products are not regulated by the United States Food and Drug Administration, raising concerns regarding product quality, consistency, and chemical composition.

Objectives: This study characterized vaping devices based on design, function, marketing features, and chemical composition.

Methods: Vaping devices were obtained between 2024-2026 through a confiscation program within the Virginia school system. Devices were categorized by brand, activation mechanism, chamber configuration, chamber capacity, puff count claims, price, prevalence, and consumer-focused features to help determine accessibility and appeal of the devices. Post-manufacturing alterations or damage were noted. Unique devices' vape formulations were screened using gas chromatography mass spectrometry (GC-MS), and quantitation was performed using liquid chromatography tandem mass spectrometry (LC-MS/MS) using previously validated and published methods.

Results: A total of 47 devices with a novel feature or design were identified. 16 multi-chamber devices were submitted during the 2024-2025 school year, and 27 devices (and counting) were submitted during the 2025-2026 school year. Devices permitted users to switch between chamber formulations, or combine them, via three main mechanisms: a toggle switch, a sliding mouthpiece, or a button to redirect the power between chamber coils. Boutiq brand devices have become more prevalent, which incorporate a "blinker" feature that can be seen as a game. Multi-chamber devices were comprised of dual-nicotine, dual-cannabinoid, "spliffs" (one nicotine and one cannabinoid chamber), triple-cannabinoid, nicotine and sweetener, and dual nicotine with a coolant chamber. Compared to historical single-chamber vapes that commonly utilized 1 to 5 mL chambers, most novel devices had substantially increased liquid capacity, as much as 30 mL, and advertised puff counts of up to 50,000. Novel features identified include: fidget-style components (Off-switch), an integrated stress ball (UT-50000), a voice-activated device with a claimed "AI Mode" (iJoy Uranus mate), LED screens with puzzles (Off-switch), a hookah-style product with integrated speakers (Oilit Hookahlit), and a device that has built-in games and can play music and make calls when connected to a phone (Airfuze). Devices ranged in price from $18.99 to $35.99. Structural damage to devices, such as charring and melting of e-liquid holding elements, was observed. Nicotine was identified in 30.4% of products (concentration ranging from <1% to 2.82%). One or more cannabinoids were identified in 69.6% of products (total cannabinoid concentrations ranged from 28.7% to 40.7%). Common cannabinoids included (in order of

prevalence): Δ9-tetrahydrocannabinol (Δ9-THC), Δ8-THC, cannabinol (CBN), cannabigerol (CBG), delta-4(8)-iso THC, cannabicitran (CBT), and Δ8-tetrahydrocannabivarin (Δ8-THCV).

Discussion: Novel vaping device prevalence has been rising and presents emerging risks to consumer health as they contribute to youth appeal and addiction. Continued surveillance is critical for determining availability, functionality, and chemical composition, as well as assessing labeling accuracy and supporting regulatory actions. Many cannabinoid products contain high concentrations of total cannabinoids, which can lead to serious adverse events, particularly for naive consumers.

Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

Conflict of Interest

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P-176

Sodium Nitrite Toxicity: Development of a Method for Rapid Toxicological Screening Using a Mobile Color-Detecting Application

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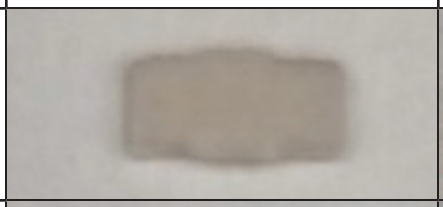
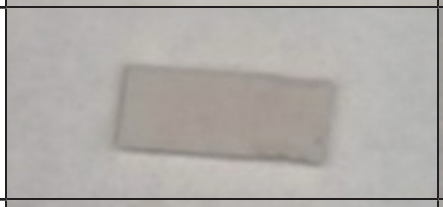
Abstract

Introduction: Sodium nitrite ($NaNO_2$) is a common, water-soluble white to yellowish powder used in pharmaceuticals, industry, and pest control. It primarily causes methemoglobinemia by disrupting the blood's ability to carry oxygen. Symptoms, ranging from headaches and dizziness to seizures, cardiac arrhythmias, or death, depend directly on the ingested amount. Clinical signs and autopsy findings in sodium nitrite poisoning are often pathologically non-specific. Therefore, prompt toxicological analysis and treatment are crucial for survival or for determining the cause of death. Currently, various methods exist for nitrite analysis. The Institute of Forensic Medicine at the Police General Hospital (IFM) employs a color test for nitrite identification, selecting the appropriate method based on the sample type. This creates a significant challenge due to matrix complexity, requiring the on-site rapid isolation of nitrite from biological samples for forensic toxicology in Thailand. Case Report: A 29-year-old Caucasian male cadaver in early decomposition presented with yellowish skin, dark fingernails, and severe visceral congestion (lungs, liver, spleen) without external wounds. The trachea contained frothy fluid, and the stomach held pink, foamy liquid. Autopsy revealed negligible coronary artery disease (5%). Toxicology screening detected nitrite in both blood and gastric contents. Concomitant therapeutic drugs—alprazolam, domperidone, haloperidol, and sertraline/norsertraline—were identified, while illicit substances, alcohol, and cyanide were absent. The findings indicate fatal acute nitrite poisoning.

Objectives: Due to the limited availability of field-test kits for nitrite in biological samples within Thailand, this study focuses on developed a novel method for rapid screening sodium nitrite detection in biological samples.

Methods: Gastric samples (0.5 mL) were acidified with 6M HCl, while blood samples (0.5 mL) were mixed with 100 mg zinc powder. Both were vortexed and tested using Griess reagent-impregnated Whatman paper; a color transition indicated a positive reaction.

Results: For the determination of sodium nitrite in gastric content samples, Method 1 proved effective. This method yielded a LOD of 200 mg/L, which was visually confirmed by a distinct purple coloration when compared to a negative control. However, this method hit a snag when applied to blood samples as the addition of acid, crucial for the reaction, caused the blood to coagulate, rendering the test unusable. Therefore, an alternative method involving the reduction reaction of zinc was developed. This approach was not only successful but also more sensitive, achieving an LOD of 100 mg/L in blood. This was indicated by a dark reddish-purple color, which was clearly visible when compared to a negative control, as shown in Table 1. Subsequent to the chemical reaction, a mobile application, developed by IFM, utilizing image analysis was used to determine the color intensity of the resulting test strips.

Concentration (mg/L)	Gastric content	Blood
0 (Negative control)		
50		
100		
200		
1000		

Discussion: This study presents a user-friendly, low-cost, and portable method for rapid sodium nitrite screening in gastric content and blood, achieving limits of detection (LOD) of 200 mg/L and 100 mg/L, respectively. Designed for forensic investigations requiring immediate results, the technique replaces visual inspection with digital colorimetric quantification via a mobile application, significantly enhancing field accuracy and usability. However, positive results still necessitate further confirmation using advanced analytical techniques.

Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

P-177

Effects of Cigarette Smoking on Direct Alcohol Biomarkers Across Biological Matrices After Controlled Alcohol Administration

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Abstract

Introduction: Cigarette smoking and alcohol use frequently co-occur, yet the effect of smoking on direct alcohol biomarkers remains poorly characterized. This is an important issue in forensic and clinical toxicology, where ethyl glucuronide (EtG), ethyl sulfate (EtS), and phosphatidylethanol (PEth) are increasingly used to assess recent or repeated alcohol exposure. Because these biomarkers are often interpreted in low or borderline concentration ranges, any smoking-related suppression may influence result interpretation. Despite previous evidence suggesting that nicotine exposure may alter alcohol absorption and blood alcohol concentration, it remains unclear whether smoking status also affects the concentrations of direct ethanol biomarkers across different biological matrices.

Objectives: This study aimed to investigate whether cigarette smoking influences the concentrations of selected direct alcohol biomarkers after controlled ethanol administration and whether this effect differs across biological matrices.

Methods: A total of 24 healthy volunteers were included, comprising 12 smokers and 12 non-smokers. Following a light standardized breakfast and a 60-min waiting period, all participants received an oral ethanol dose of 0.7 g/kg as vodka. Blood alcohol concentration (BAC) was measured in whole blood by HS-GC-FID. EtG, EtS, and PEth were determined by LC-MS/MS in whole blood, urine, dried blood spots (DBS), and dried saliva spots (DSS) at predefined time points. Group comparisons were primarily performed using non-parametric analyses. Additional adjusted models were used to evaluate whether observed differences persisted after controlling for BAC, sampling time, and sex. Matrix-specific smoker/non-smoker suppression ratios and correction factors were estimated using regression-based models.

Results: Mean BAC was significantly lower in smokers than in non-smokers ($p < 0.001$). In unadjusted comparisons, smokers showed significantly lower concentrations of DBS-PEth 16:0/18:1 ($p = 0.008$), DBS-EtG ($p < 0.001$), urinary EtS ($p = 0.048$), urinary EtG ($p = 0.002$), and DSS-PEth 16:0/18:1 ($p = 0.027$). Whole-blood PEth 16:0/18:1 was also lower in smokers, although the difference was borderline ($p = 0.169$). In adjusted analyses, BAC did not independently explain biomarker concentrations. The strongest smoking-associated suppression was observed for urinary biomarkers, with smoker/non-smoker ratios of 0.30 for EtG and 0.40 for EtS, corresponding to correction factors of 3.38 and 2.53, respectively. DBS-EtG showed moderate suppression (ratio 0.70), whereas DBS-EtS showed no meaningful smoking effect. For PEth 16:0/18:1, the effect was matrix-dependent, with substantial suppression in DSS (ratio 0.57), borderline suppression in whole blood (ratio 0.51), and minimal suppression in DBS (ratio 0.94).

Discussion: These findings provide evidence that cigarette smoking may alter direct alcohol biomarker concentrations in a matrix-specific manner. The strongest suppressive effect was observed for urinary EtG and EtS, while DBS-based biomarkers appeared less affected, particularly DBS-EtS and DBS-PEth. The study is novel combining controlled alcohol administration with parallel multi-matrix biomarker assessment to examine smoking as a potential interpretive confounder. These results may be particularly relevant in forensic and clinical evaluation of low or borderline biomarker findings and support the need for larger studies to refine interpretation frameworks for alcohol biomarkers in smokers.

Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

P-178

Effects of Cyclodextrins on Cannabidiol Solubility and Stability in Simulated Gastric Fluid

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Abstract

Introduction: Cannabidiol (CBD) has attracted attention as an ingredient in pharmaceuticals and health foods, but its poor aqueous solubility requires formulation-based solubilization, as exemplified by the oily vehicle used in Epidiolex. However, CBD is known to be converted into Δ^9-THC under acidic conditions. Therefore, formulation approaches that increase CBD solubility should also consider gastric stability, because increased solubility may increase the amount of CBD available for acid-catalyzed conversion in gastric fluid, leading to Δ^9-THC formation in the stomach. From a forensic toxicological perspective, Δ^9-THC formed from CBD in the stomach cannot be analytically distinguished from orally ingested Δ^9-THC, which may complicate the interpretation of Δ^9-THC detection after CBD product consumption. Accordingly, CBD formulations should be designed to improve CBD solubility while suppressing gastric Δ^9-THC formation. In this study, we focused on cyclodextrins (CDs), which have hydrophobic cavities and can form inclusion complexes, as a possible approach to achieving both CBD solubilization and suppression of acid-catalyzed conversion.

Objectives: This study aimed to evaluate the solubilizing effects of various CDs and the stability of CBD-CD complexes under acidic conditions using simulated gastric fluid.

Methods: An excess amount of CBD was added to aqueous solutions of α-CD, γ-CD, sulfobutylether-β-CD (SBE-β-CD), 2-hydroxypropyl-β-CD (HP-β-CD), or 2,6-di-O-methyl-β-CD (DM-β-CD) at 0–40 mM. After 12 h, insoluble CBD was removed by filtration, and CBD concentrations in the filtrates were quantified by LC-MS/MS using CBN as an internal standard. For the stability test, CD-containing solutions were prepared to contain 100 ppm dissolved CBD, mixed with simulated gastric fluid (pH 1.2), and shaken at 37°C for 4 h. After neutralization and extraction, the percentage of dissolved CBD converted to Δ^9-THC was quantified by GC/MS. The CD-free control was performed using 0.1 ppm dissolved CBD, which corresponded to the aqueous solubility of CBD without CD.

Results: Among the examined CDs, β-CD-based derivatives appeared to provide the most suitable cavity size for CBD inclusion. In the absence of CD, the aqueous solubility of CBD was 0.1 ppm, whereas 40 mM SBE-β-CD, HP-β-CD, and DM-β-CD increased the apparent CBD concentrations to 218, 125, and 766 ppm, respectively. In the stability test using simulated gastric fluid, 65.0% of dissolved CBD was converted to Δ^9-THC in the absence of CD, based on a dissolved CBD concentration of 0.1 ppm, whereas the corresponding percentages with SBE-β-CD, HP-β-CD, and DM-β-CD were 15.9%, 3.9%, and 0.76%, respectively, based on a dissolved CBD concentration of 100 ppm.

Discussion: DM-β-CD showed the highest solubilizing effect on CBD among the examined CDs and was also the most effective in suppressing the acid-catalyzed conversion of CBD to Δ^9-THC. This may be due to stronger hydrophobic interactions between CBD and the methyl-substituted β-CD cavity, resulting in more stable inclusion of CBD. These findings indicate that appropriate CD selection can improve CBD solubility while limiting gastric Δ^9-THC formation. By suppressing gastric Δ^9-THC formation associated with CBD solubilization, DM-β-CD can reduce interpretative uncertainty regarding the origin of Δ^9-THC detected after CBD product consumption, thereby contributing to a clearer forensic toxicological interpretation of whether cannabinoid findings reflect oral Δ^9-THC intake or gastric conversion of CBD.

Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

P-179

Evaluating Synthetic Melanins for Benzodiazepine Uptake: Potential Forensic Applications

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Abstract

Introduction: Benzodiazepines are among the most widely prescribed psychoactive drugs worldwide and are frequently involved in misuse and intoxication. Their extensive therapeutic and non-medical use makes them highly relevant targets in forensic toxicology, particularly in urine drug testing and confirmatory analyses. However, their detection and quantitation in biological matrices remains analytically challenging due to low concentrations, matrix complexity, interferences, and the need for efficient sample clean-up and preconcentration strategies. Conventional extraction methods may present limitations in selectivity, recovery, and sustainability. Moreover, the known affinity of many drugs for melanin-rich biological matrices (e.g., hair) suggests that melanin-based materials may offer a biomimetic approach for the selective uptake of these compounds. In this context, synthetic melanins may represent promising adsorbent materials for the selective uptake, extraction, and recovery of benzodiazepines from biofluids, with possible application in forensic sample preparation and toxicological workflows.

Objectives: This study aims to evaluate the potential application of two synthetic melanins—DA-melanin, mimicking eumelanin found in human skin, and DHN-melanin, mimicking melanin structures typical of fungi and plants—for the removal of nine commonly used benzodiazepines and to preliminarily assess their performance in urine samples spiked with benzodiazepines.

Methods: Batch adsorption experiments were conducted by incubating benzodiazepine-spiked solutions with melanin-based materials in Falcon tubes under continuous mixing (40 rpm, 24 h) to assess uptake efficiency, while kinetic studies were performed to investigate adsorption mechanisms. A mixture of nine benzodiazepines at low-level concentrations (10-50 µg L^{-1}) was used, and analytes were quantified by LC-MS/MS. Adsorption kinetics were modelled using pseudo-first-order (PFO) and pseudo-second-order (PSO) equations. In addition, magnetic melanin-based materials and melanin-coated filters (polyethersulfone, PES, and nylon) were evaluated through syringe filtration experiments to assess their potential for benzodiazepine uptake.

Results: Compound-dependent adsorption behaviour was observed for the investigated compounds. A marked difference in adsorption performance was observed between the two materials: DA-melanin showed limited adsorption, whereas DHN-melanin exhibited substantially higher and faster uptake for most investigated benzodiazepines, with near-complete adsorption observed for selected analytes already within the first minutes of exposure. In aqueous experiments, DHN-melanin showed near-complete uptake for several analytes, particularly zolpidem, bromazepam, alprazolam, and diazepam. Melanin-coated nylon filters retained adsorption performance over up to three filtration cycles. Based on preliminary adsorption results, DHN-melanin was selected for further evaluation in drug-spiked urine samples using both batch adsorption experiments and melanin-coated nylon filters. Adsorption efficiencies ranged approximately from 35–95% depending on analyte and material configuration. Higher uptake was generally observed for zolpidem, clonazepam, and diazepam, whereas lower

adsorption was found for lorazepam and oxazepam, supporting the applicability of melanin-based materials in biological matrices.

Discussion: The results demonstrate the potential of synthetic melanins as sustainable adsorbent materials for the removal of benzodiazepines. The higher performance of DHN-melanin suggests that structural features of melanin strongly influence adsorption behavior, likely affecting interactions with aromatic and heterocyclic pharmaceutical compounds. The integration of magnetic materials and melanin-coated filters suggests their potential applicability in sample preparation strategies for benzodiazepine analysis. These findings open new perspectives for the use of melanin-based materials in forensic toxicology, particularly for the preconcentration and analysis of drugs in complex biological matrices.

Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

P-180

Investigation of the Thermal Conversion of Cannabidiol (CBD) to Δ9-Tetrahydrocannabinol (THC) in CBD-containing Smoking Products Using Pyrolysis-GC/QTOF-MS

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Abstract

Introduction: In Japan, the consumption and possession of Δ^9-tetrahydrocannabinol (Δ^9-THC) and its psychoactive analogues are criminalized. Meanwhile, other compounds found in the cannabis plant such as cannabidiol (CBD) remain legal but recent changes in legislation designated the non-psychoactive cannabinol (CBN), as an illegal substance. Nonetheless, there has been an increase in number of smoking products containing CBD sold in Japan marketed as 'THC-free' to address consumer concerns. Nonetheless, there is some evidence that suggest that CBD isomerizes to Δ^9-THC within the operating temperatures of electronic cigarettes typically starting at 250°C. In contrast, direct isomerization of CBD to CBN is not widely reported but may be formed from oxidative degradation of THC.

Objectives: The aim of this study was to observe and quantify the thermal isomerization of CBD to Δ^9-THC in an oxygen-free environment using pyrolysis-GC/QTOF-MS. A secondary aim was to observe whether CBN may be formed from CBD isomerization.

Methods: Five commercially available CBD-containing heat-sticks and two CBD vape products were selected by convenience sampling. Pyrolysis-gas chromatography quadrupole time-of-flight mass spectrometry (Py-GC/QTOF-MS) was performed using a multi-shot pyrolyzer (EGA/PY-3030D, Frontier Laboratories) interfaced to a GC/QTOF system (8890A/7250QTOF, Agilent Technologies) and was operated in full scan mode. Aliquots of 1µL sample or 10mg of solid sample were flash pyrolyzed for 1 minute at 250, 300, 350, 400, and 500 °C in a helium gas environment. Compound identification was carried out by spectral matching against powdered CBD and CBN; synthesized THC and crossed checked with the Wiley 20 and NIST 20 mass spectral libraries

Results: Characteristic mass fragment patterns of Δ^9-THC (m/z 299.1855 and 314.2062) but not Δ^8-THC formation (*m/z* 231.1388 and 314.2071) were observed in four out of seven CBD-containing products. Δ^9-THC generation peaked at approximately 400 °C, with no appreciable increase at higher temperatures. The peak intensity of Δ^9-THC was approximately two orders of magnitude lower than that of the parent CBD, indicating that conversion efficiency is modest, though Δ^9-THC was consistently detected above the limit of detection (LOD) of 0.1ppm. No detectable levels of CBN (*m/z* 295.1561 and 310.1763) were found in the samples. CBD to Δ^9-THC conversion was not detected in CBD powder products.

Discussion: These results indicate that partial conversion of CBD to Δ^9-THC can occur in certain CBD-containing products under thermal conditions consistent with electronic cigarette operation. The absence of conversion in powder formulations suggests that product matrix composition, may play a significant role in facilitating this thermal isomerization in an oxygen-free environment. These findings may have implications for consumers in zero tolerance jurisdictions globally and more needs to be investigated.

Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

P-181

Validation of the Rudolph AutoFlex® R827 for Automated Human Urine pH and Specific Gravity Testing

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Abstract

Introduction: Federal workplace urine drug testing, governed by the Substance Abuse and Mental Health Services Administration (SAMHSA), mandates Specimen Validity Tests (SVT) to ensure sample integrity. SVT detects potential tampering by checking for adulteration, substitution, or dilution using key parameters including pH, specific gravity, creatinine concentration, and the presence of oxidants. While creatinine and oxidant analyses are typically automated, the required confirmation for pH and specific gravity is often still labor-intensive manual processes. Here, we present the first validated automated method in our laboratory, complied with National Laboratory Certification Program (NLCP), for both initial and confirmatory pH and specific gravity analyses using the Rudolph AutoFlex® R827 Toxicology Lab automation system.

Objectives: This study aimed to conduct a comprehensive validation of the Rudolph AutoFlex® R827 to determine its suitability as an accurate, reliable, and efficient replacement for the laboratory's established manual methods for pH and specific gravity testing.

Methods: The validation assessed instrument performance across several key parameters for both pH (ranging from 3.0 to 12.0) and specific gravity (ranging from 1.0000 to 1.0230). Precision was examined using five replicates of standards, requiring a coefficient of variation (CV) below 10%. Accuracy was required to be within ±0.2 units of the target value for pH measurement and, for specific gravity, ±0.0002 for the 1.0000 control and ±0.0004 for other controls. Sample carryover was evaluated by analyzing samples immediately following low- or high-concentration samples, which could potentially affect the accuracy of the results. Finally, a comparability study was performed by analyzing over 40 authentic specimens on both the AutoFlex® R827 and the incumbent manual instruments (Mettler Toledo pH meter and Rudolph J257 refractometer).

Results: The AutoFlex® R827 was found to be capable of meeting all the validation criteria. The instrument demonstrated exceptional precision with the CVs for pH and specific gravity of < 1.6% and < 0.01%, respectively. All accuracy measurements were within the acceptance range. Sample carryover was determined to be negligible. The parallel study confirmed a strong consistency with the results produced with the manual methods, yielding r^2 values of 0.9962 for pH and 0.9942 for specific gravity, thereby demonstrating excellent method comparability.

Discussion: The Rudolph AutoFlex® R827 is a robust and reliable automated testing system for high efficiency pH and specific gravity analyses. The instrument can interface the commonly employed LIMS. Our validation data confirmed that the instrument offers precise and accurate results consistently comparable to the established manual techniques, without significant sample-to-sample carryover. Implementation of the AutoFlex® R827 would effectively eliminate a critical laboratory workflow bottleneck, enhance sample throughput, and improve production efficiency while upholding the rigorous standards of forensic toxicology.

Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

P-182

Identification of a UGT2B7 Residue Which Determines Glucuronidation at the 6-OH Position of Morphine, and Species Differences in This Residue and Morphine Glucuronidation

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Abstract

Introduction: It has been thought that the functions of ortholog UDP-glucuronosyltransferase (UGT) isoforms in rats and mice are similar. Rat UGT2B1 (rUGT2B1) is known to catalyze morphine-3-glucuronidation. However, we previously found that mouse Ugt2b1 (mUgt2b1) has little activity toward morphine. We also found that mouse Ugt2b36 (mUgt2b36) is the predominant isoform involved in morphine glucuronidation in mice.

Objectives: In our preliminary studies, swapping the substrate determination domain between rUGT2B1 and mUgt2b1 resulted in exchanged morphine glucuronidation activity. Further, exchanging the amino acid at position 117, Phe in rUGT2B1 and Ser in mUgt2b1, also exchanged morphine glucuronidation activity. mUgt2b36, which glucuronidates morphine, possesses Phe117. The major morphine glucuronidating enzyme in humans is UGT2B7. Unlike rUGT2B1 and mUgt2b36, human UGT2B7 glucuronidates morphine at the 6-OH group , in addition to the 3-OH group. In UGT2B7, Met116 is the amino acid corresponding to Phe117 in rUGT2B1. Therefore, we hypothesized that Met116 of UGT2B7 is involved in determining substrate specificity for morphine-6-glucuronidation. Thus, we constructed two mutants replacing Met116 in UGT2B7 either with the corresponding Phe117 in rUGT2B1 or Ser117 in mUgt2b1.

Methods: We used the baculoviral-Sf9 expression system for expression of the mutants. The mutants were built by site-directed mutagenesis using UGT2B7 as template. The activity of the mutants was assayed with 5 mM morphine. Morphine-3- and 6-glucuronides were measured by HPLC equipped with a fluorescent detector.

Results: Substitution of UGT2B7 Met116 with Ser abolished morphine-3-glucuronidation activity while morphine-6-glucuronidation was retained. In contrast, changing Met116 to Phe resulted in three-times higher rates toward morphine-3-glucuronidation than the wild-type but abolished morphine-6-glucuronidation activity.

Discussion: These results suggest that Met116 of UGT2B7 determines the 3-OH and 6-OH-glucuronidation of morphine. These results help explain the differences in morphine glucuronidation activity between rodents and humans. This has the potential to be applied in practice as a functional species-discriminating marker.

Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

P-183

Evaluating Post-Analytical Stability of Blood Ethanol Samples Using Headspace-GC-FID

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Abstract

Introduction: The analysis and determination of blood alcohol concentration (BAC) is a cornerstone test in forensic toxicology. Over the years, many laws and policies have been established to promote safe use of ethanol and to protect the general public against the dangers of alcohol abuse. Thus, there is an increasing demand for alcohol testing, both with driving while intoxicated and post-mortem investigations, to provide scientific evidence for criminal charges or to assist pathologists in determining a cause of death, respectively. The majority of recent blood ethanol research focuses on pre-analytical stability, newer instrumentation and advanced methodologies for evaluating blood ethanol. Pre-analytical storage and conditions in respect to stability of ethanol in blood samples have been researched extensively with multiple authors determining that measured BAC decreases slightly over time during storage at room temperature or refrigerated. However, research assessing the stability of measured BAC in post-analytical headspace vials is lacking. Due to the volatile nature of the compounds being measured, it is widely believed that a headspace sample can only be tested once accurately. Under this belief, any unsuccessful runs or sample results would require a full setup from start with additional sample consumed.

Objectives: The purpose of this research was to determine the stability of blood ethanol obtained from re-injected vials via Agilent dual column headspace-GC-FID.

Methods: Over 6 months and 22 individual batch analyses, 303 samples were prepared and analyzed using Headspace-GC-FID for the presence of blood ethanol using 0.02% n-propanol as an internal standard. Each individual batch was re-injected after storage at room temperature for 72 hours to mimic a typical weekend absence. Ethanol signal response, n-propanol signal response and measured blood alcohol concentration were evaluated for stability in terms of percent change. Sample population consisted of negative human blood fortified with known concentrations of ethanol via certified reference material purchased from accredited vendors (n=187) in the form of calibrators and controls, as well as unknown post-mortem and ante-mortem blood samples submitted to the Nassau County Medical Examiner's Office Department of Forensic Toxicology (n=116). All samples were tested using similar conditions and internal standard. A simple dependent T-test was used to compare the blood alcohol concentrations, internal standard signal response, and ethanol signal response between the initial measurements and the re-injected measurements.

Results: Significant differences were observed in the signal response for both n-propanol ($p<.001$) and ethanol ($p<.001$) between the two test groups however, there was a non-significant difference in measured BAC between the two groups $t(302) = 0.3$, $p = 0.780$, $d = 0.016$.

Discussion: Results suggest very little effect of re-injection after 72-hour storage at room temperature on measured blood ethanol concentration using headspace GC-FID. While there was significant loss of ethanol and internal standard on reinjection, the calculated ethanol

concentration was unaffected due to the similar properties of both volatile measurands as well as the consistency of the response ratio of ethanol:n-propanol in reinjected vials. In addition, observed differences in measured BAC on re-injection were well within the NCME's measurement uncertainty of 11% at k=3.

Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

P-200

THC Isomers & Derivatives & Homologs, Oh My! – A Chromatographic Interference Study by UPLC-MS/MS Involving 45 Tetrahydrocannabinol Analogs

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Abstract

Introduction: Tetrahydrocannabinol (THC) isomers, derivatives, and homologs, collectively known as THC analogs, are psychoactive compounds structurally related to Δ^9-tetrahydrocannabinol (Δ^9-THC). They are often produced from hemp-derived cannabidiol via isomerization. Given the increased prevalence of novel THC analogs present in toxicology specimens in recent years following the reclassification of hemp in the 2018 Farm Bill, an expanded chromatographic interference study was warranted to determine if the presence of THC analogs interfere with the proper identification and/or quantitation of Δ^9-THC, carboxy-Δ^9-THC, and/or 11-OH-Δ^9-THC in toxicology casework.

Objectives: A total of 45 THC analogs were procured from various manufacturers and evaluated by Ultra-Performance Liquid Chromatography Tandem Quadrupole Mass Spectrometry (UPLC-MS/MS) to determine which, if any, interfere with the identification and/or quantitation of Δ^9-THC and its metabolites, 11-nor-9-carboxy-Δ^9-tetrahydrocannabinol (carboxy-Δ^9-THC) and 11-hydroxy-Δ^9-tetrahydrocannabinol (11-OH-Δ^9-THC). Based on the outcome of the study, disseminate further guidance to personnel regarding any observed chromatographic interferences affecting the identification and/or quantitation of Δ^9-THC, carboxy-Δ^9-THC, and/or 11-OH-Δ^9-THC and any resulting method limitations due to the possible presence of one or more interfering analogs.

Methods: 45 THC analogs were each evaluated in solvent using a Waters Acquity H-Class UPLC equipped with a Waters BEH C18 1.7 µm, 2.1 x 75 mm column and Waters TQS Micro detector operating in MRM mode. The laboratory has two existing, previously validated UPLC-MS/MS methods for cannabinoid identification and quantitation – standard gradient (11.5 minutes) and long gradient (19.5 minutes). Both methods were used throughout the study. The THC analogs were first analyzed in solvent (50:50 deionized water to acetonitrile) utilizing the standard gradient. If baseline-to-baseline resolution of Δ^9-THC, carboxy-Δ^9-THC, and/or 11-OH-Δ^9-THC was not achieved on the standard gradient, then the interfering analogs were spiked at various concentrations into a separate set of method quality controls containing various, known amounts of Δ^9-THC, carboxy-Δ^9-THC, and 11-OH-Δ^9-THC and re-analyzed on both the standard and long gradients. A table was generated detailing all THC analogs evaluated and any chromatographic interferences exhibited during the study.

Results: Of the 45 THC analogs studied, four analogs (exo-THC, Δ^8-THC, carboxy-Δ^8-THC, and 11-OH-Δ^8-THC) exhibited the greatest impact on the identification and/or quantitation of Δ^9-THC and its metabolites, carboxy-Δ^9-THC and 11-OH-Δ^9-THC, by UPLC-MS/MS analysis. As the concentration ratio of exo-THC:Δ^9-THC, Δ^8-THC:Δ^9-THC, carboxy-Δ^8-THC:carboxy-Δ^9-THC, and/or 11-OH-Δ^8-THC:11-OH-Δ^9-THC increased in individual specimens, chromatographic resolution decreased. Identification and/or quantitation of Δ^9-THC, carboxy-Δ^9-THC, and 11-OH-Δ^9-THC was compromised with an increasing likelihood relative to the concentration of exo-THC, Δ^8-THC, carboxy-Δ^8-THC, and 11-OH-Δ^8-THC.

Discussion: Exo-THC, Δ^8-THC, carboxy-Δ^8-THC, and 11-OH-Δ^8-THC are distinguishable from Δ^9-THC, carboxy-Δ^9-THC, and 11-OH-Δ^9-THC on existing UPLC-MS/MS methods due to differences in retention times, so there is no risk of misidentification. Thus, no method changes were immediately required and a review of historical casework was not warranted. However, depending on the concentration ratios of exo-THC:Δ^9-THC, Δ^8-THC:Δ^9-THC, carboxy-Δ^8-THC:carboxy-Δ^9-THC, and/or 11-OH-Δ^8-THC:11-OH-Δ^9-THC present in individual specimens, acceptable chromatographic resolution may or may not be achievable. Therefore, the approach to UPLC-MS/MS data review and evaluation was revised to mitigate any risk of inaccurate reporting. Future investigation is warranted to determine if baseline-to-baseline resolution can be achieved via column and/or gradient modifications.

Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

P-201

Development of a Quantitative Method for Opioids in Blood using LC-MS/MS and Filter Vials

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Abstract

Introduction: Houston Forensic Science Center (HFSC) previously used two liquid chromatography tandem mass spectrometry (LC-MS/MS) methods for confirmatory analysis of a panel of opioids and related compounds, including morphine, oxymorphone, hydromorphone, codeine, oxycodone, hydrocodone, tramadol, o-desmethyltramadol, 6-acetylmorphine, buprenorphine, norbuprenorphine, fentanyl, norfentanyl, methadone, and 2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine (EDDP). However, the existing methods showed carryover with buprenorphine, methadone, and EDDP, requiring washes between every sample, and the calibration curves fluctuated between linear and quadratic models, complicating consistent passing and verification of calibrators (cals) and quality control (QC) samples.

Objectives: This project aimed to consolidate the previous methods into a single assay and to develop an optimized extraction to enhance analytical performance, efficiency, and reliability for casework. Method optimization included developing a streamlined extraction procedure, expanding the calibration range to encompass concentrations relevant to HFSC casework, optimizing cal/QC concentrations across the range, and increasing internal standard concentrations to maintain consistent calibration curve models.

Methods: Cals/QCs were prepared through serial dilution in methanol from stock solutions. From these dilutions, 30 µL was fortified with 30 µL of internal standard into 300 mL of blank blood. The samples were centrifuged following a protein precipitation with 380 mL of cold acetonitrile. The supernatants were then transferred to Thompson filter vials. The filtered supernatants were transferred into conical tubes, evaporated, and reconstituted with 60 µL of a 95:5 mixture of mobile phase A (water with 0.1% formic acid) and mobile phase B (methanol with 0.1% formic acid). The samples were then analyzed using LC-MS/MS analysis. LC method initial parameters began at 95% A, decreasing to 2% A at 7.50 minutes, which was held for one minute, then ramped back up to the starting conditions at 8.60 minutes and held for 10 minutes with a flow of 0.7 mL/min.

Results: The previous methods required a total run-time of 13.0 minutes and 9.6 minutes, whereas the consolidated method achieved a reduced run-time of 10.0 minutes. Additionally, sample volume requirements decreased from a total of 2 mL of blood to 300 µL, significantly improving sample efficiency. This streamlined method also uses only one organic mobile phase while the prior methods used two with more additives. Inter-sample wash steps were eliminated to improve throughput, and dichloromethane was removed from the extraction procedure to reduce hazardous solvent use, thereby limiting analysts' exposure. Collectively, these improvements decreased overall run-time while maintaining the robustness required for forensic toxicology analysis.

Discussion: This work presents a streamlined LC-MS/MS method employing filter vial technology for the rapid quantification of 15 opioids in blood. The expanded calibration range for 6-acetylmorphine, codeine, morphine, oxycodone, tramadol, hydrocodone, EDDP, methadone, and o-desmethyltramadol is expected to reduce the need for sample dilution. Furthermore, comparison of historical and combined-method results will help confirm consistent detection and opioid trends. Additionally, it is expected that operational evaluation will demonstrate clear cost and efficiency benefits, including reduced reagent consumption and analyst time. Overall, this method provides a practical and progressive solution for HFSC, supporting improved turnaround times and enhanced safety while maintaining high standards of analytical quality.

Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

P-202

Evaluating DART-QQQ-MS for Comprehensive Forensic Toxicology Sample Screening

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Abstract

Introduction: The 2005 debut of direct analysis in real time tandem mass spectrometry (DART-QQQ-MS) as the first commercially available open-air, ambient ion source highlighted its lack of sample preparation, versatility, and rapid analysis time. The two-step ionization process is summarized by the formation of helium metastable species and subsequent analyte protonation via Penning and proton transfer mechanisms. Success with this technique has been demonstrated across multiple forensic disciplines, though relevant research has largely concentrated on identification of seized drug materials and urine-based screening. With limited analyte restrictions and high accuracy through its tandem mass spectrometry interface, DART-QQQ-MS may provide reliable qualitative data during a forensic toxicology workflow.

Objectives: This study sought to investigate the potential of DART-QQQ-MS for large-scope rapid toxicology screening. Emphasis was placed on method optimization, analyte-specific performance, and practical detectability in common biological matrices.

Methods: 24 analytes were included in this analytical method, comprising traditional opioids e.g., morphine), NPS opioids (e.g., carfentanil), and common adulterants (e.g., xylazine). Fentanyl-d5 was used as the internal standard. The qualitative range was assessed from 0.1-500 ng/mL in blood and urine. Biological samples (0.5 mL) were prepared using a basic liquid-liquid extraction. Analysis was conducted using a Bruker EVOQ DART-TQ+. Method optimization was performed using various control mixtures in methanol (100 ng/mL) and confirmed with extracts from spiked blood and urine (both 100 ng/mL) for the following parameters: 1) DART gas (He) temperature; 2) cone temperature; 3) Injection Mode (JumpShot, Linear, Circular); 4) Scan time (30-4ms); 5) Reconstitution composition.

Results: An opioid-focused qualitative panel was developed using multiple reaction monitoring with limits of detection ranging from 0.5-25 ng/mL in blood and urine. Highly basic, lipophilic analytes such as the fentanyl analogs were less prone to matrix effects, resulting in stable, low detection limits post extraction. Key findings for optimization include sufficiently high DART gas and cone temperature pairing to ensure desorption and induction into the ion flight path (e.g., 300/300˚C), mid-range scan time (20-10ms, linear injection), and reconstitution with 50 uL of MeOH:ACN (50%:50%). Analytical performance was inconsistent between analytes, such as concentration-dependent ion ratio behavior and varied sensitivity. Notably, at otherwise identical conditions, analyte recovery decreased as the complexity of samples increased, suggesting competitive ionization effects.

Discussion: DART-QQQ-MS was not suitable for analyzing a large, structurally diverse scope in blood or urine extracts. Performance was challenged by variability due to class differences, ionization effects, and sample composition. A narrowed scope concentrating on opioids and adulterants was evaluated, allowing for targeted optimization to mitigate variable analyte-specific performance. Naturally, there was limited ability to differentiate isomers due to the absence of chromatographic separation. These factors could present challenges for NPS casework, where differentiation of closely related analogs is often critical. Additionally, poor within-run precision increases the risk of low-level false negatives. Despite these limitations, the method's speed, green-chemistry initiatives, and strong qualitative performance for many opioids position DART-QQQ-MS as a potential complementary screening tool in narrow-scope toxicology assays.

Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

P-203

Immunoassay for the Detection of Zolpidem in Urine on the Beckman Coulter AU480 Clinical Analyzer

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Abstract

Introduction: Zolpidem (Ambien®) is a non-benzodiazepine sedative-hypnotic drug prescribed to treat insomnia. Despite its intended use as a short-term treatment, it steadily became the subject of misuse and abuse cases. Reported cases include consumption exceeding recommended dosages and failure to adhere to prescribing instructions, which have been linked to adverse outcomes such as impaired cognition, accidents, and injury. More severe consequences have also been documented, including suicide attempts and its involvement in drug-facilitated sexual assault (DFSA) and drug-facilitated crimes (DFC).

Objectives: The purpose of this study was for Lin-Zhi International, Inc. (LZI) to develop an enzyme immunoassay to reliably and accurately detect zolpidem and its metabolites in urine to address the rise in the above mentioned cases.

Methods: The LZI Zolpidem assay was developed and evaluated by a series of studies consisting of linearity, precision, limit of detection, cross reactivity, carryover, method comparison, and stability studies. The studies were performed qualitatively and semi-quantitatively and tested with a zolpidem cutoff of 10 ng/mL.

Assay precision was determined by spiking zolpidem into pooled negative human urine at concentrations of 0%, ±25%, ±50%, ±75%, and ±100% of the cutoff concentration and testing those samples for 22 non-consecutive days. Results were concluded by calculating the standard deviation (SD) and the percent coefficient of variation (%CV).

A cross-reactivity study was conducted to assess the assay's interference with zolpidem and its two major metabolites: zolpidem phenyl-4-carboxylic acid and zolpidem 6-carboxylic acid, as well as 115 structurally unrelated compounds, including similarly classified insomnia treatments.

A method comparison study was performed to determine the accuracy and reliability of the assay by testing 340 zolpidem-containing urine samples and comparing the results against an established detection method such as Liquid Chromatography Mass Spectrometry (LC/MS).

Results: The LZI Zolpidem assay exhibited robust precision as characterized by the SD and %CV. Within-run precision produced SD values between 2.4 mAU and 3.6 mAU and %CV values between 0.4% and 0.8%. Total precision had a range of 2.8 mAU to 3.9 mAU and %CV values of 0.6% to 0.8%.

Cross-reactivity demonstrated a 100.0% cross with zolpidem, 71.4% cross with zolpidem phenyl-4-carboxylic acid, and no cross-reactivity with zolpidem 6-carboxylic acid. Out of the 115 structurally unrelated compounds tested, the assay only encountered interference with AB-PINACA pentanoic acid at 40,000 ng/mL, JWH-018 N-5 hydroxypentyl metabolite at 45,000 ng/mL, and sertraline at 80,000 ng/mL.

The method comparison study showed over 95% positive agreement and 100% negative agreement. Discrepant samples were found only in the upper near cutoff range (+50%).

Discussion: The assay displayed accurate and reliable results on the Beckman Coulter AU480 clinical analyzer. Method comparison testing of clinical samples showed strong agreement between the LZI Zolpidem assay and LC/MS confirmation method. Despite the presence of some interfering compounds, the concentrations encountered are higher than the established lethal doses for those compounds, ensuring that the assay is still reliable.

Disclosure

Yes, I, or a member of my immediate family, has a financial interest to disclose.

Conflict of Interest

Employee of Lin-Zhi International, Inc.

P-204

Redevelopment of a High-specificity Homogeneous Immunoassay for Ethyl Glucuronide (EtG) at Dual Cutoffs

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Abstract

Introduction: Ethyl glucuronide is a metabolite of alcohol produced in the liver and excreted in the urine. It is a highly sensitive biomarker, making it an ideal indicator of ethanol usage for situations that require alcohol abstinence; common use cases include professional monitoring or alcohol treatment.

Objectives: The objective of this study is to achieve greater specificity and sensitivity compared to the previous assay.

Methods: The Lin-Zhi International, Inc. (LZI) Ethyl Glucuronide Enzyme Immunoassay was designed for use at 200 and 500 ng/mL cutoff concentrations. The following studies were performed: method comparison, cross reactivity, and precision. All studies were tested on the Beckman Coulter AU480 automated clinical analyzer.

For precision testing, negative human urine was spiked with ethyl glucuronide to create samples at 200 and 500 ng/mL cutoff concentrations, and at ±25%, ±50%, ±75%, and ±100% of both cutoffs. Samples were analyzed in duplicates over 22 non-consecutive days with two runs per day, totaling 88 replicates per concentration to assess within-run and total precision.

A method comparison study was conducted by testing 326 unaltered human clinical samples with the LZI Ethyl Glucuronide assay and comparing results against a Liquid Chromatography / Mass Spectrometry (LC/MS) confirmation method.

For cross-reactivity testing, ethyl glucuronide and other structurally-related compounds were individually spiked into pooled negative urine aliquots and tested with the assay. The lowest concentration of each compound producing a positive result equivalent to both cutoffs were determined to calculate the percent cross-reactivity.

Results: For the precision study at the 200 ng/mL cutoff, total qualitative precision had a %CV range of 2.2% to 8.2%. The precision within-runs at this concentration had a %CV range of 1.6% to 6.6%. When run at the 500 ng/mL cutoff, the total qualitative precision had a %CV range of 1.8 to 7.4%; within run precision calculations resulted in a %CV range of 1.0 to 5.1%.

The method comparison study between the LZI Ethyl Glucuronide assay and LC/MS results demonstrated over 95% agreement for both positive and negative samples at each cutoff concentration.

The cross-reactivity testing showed 100% concordance with ethyl glucuronide and no cross reactivity with the following alcohol metabolites: acetaldehyde and ethyl sulfate. Three structurally related compounds caused interference: isopropyl-β-D-glucuronide at 7,600 ng/mL (200 cutoff) and 18,050 ng/mL (500 cutoff), methyl-b-D glucuronide at 7,000 ng/mL (200 cutoff) and 16,000 (500 cutoff), and n-propyl-b-D glucuronide at 1,960 ng/mL (200 cutoff) and 5,390 ng/mL (500 cutoff).

Discussion: The LZI Ethyl Glucuronide Immunoassay demonstrated consistent and reliable results on the Beckman Coulter AU480 analyzer. Precision testing showed low variability across replicate runs, indicating robust and reproducible performance. Method comparison testing of clinical samples showed strong agreement between the LZI Ethyl Glucuronide assay and LC/MS confirmation method. Cross-reactivity testing showed strong correlation with ethyl glucuronide, supporting effective detection of this analyte in urine.

Disclosure

Yes, I, or a member of my immediate family, has a financial interest to disclose.

Conflict of Interest

Employee of Lin-Zhi International, Inc.

P-205

From Trace to Truth-A.S.A.P.! Drug Detection Can't Wait: Unknown Drug Analysis Using the RADIAN™ ASAP-MS

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Abstract

Introduction: Testing suspected drug paraphernalia collected at terminal event scenes can provide valuable information in medicolegal death investigations. While not traditionally within the scope of postmortem toxicology laboratories, the Miami-Dade Medical Examiner (MDME) Toxicology Laboratory routinely analyzes confiscated evidence to include pills, powders, paraphernalia, plant material, liquids, and candies. Although findings from these materials do not directly determine cause and manner of death, the results may guide analytical testing and provide context regarding potential drug use around the time of death.

Objectives: The purpose of this presentation is to demonstrate that the Waters Corporation RADIAN™ Atmospheric Pressure Solids Analysis Probe Mass Spectrometry system (RADIAN™ ASAP-MS) is an effective and efficient tool for the evaluation of drug evidence.

Methods: The laboratory uses a "dilute-and-shoot" approach for evidence analysis, whereby samples are dissolved in methanol and injected into a Gas Chromatography Mass Spectrometry system (GC-MS). For this study, ten previously tested samples were prepared using the same approach and reanalyzed using the RADIAN™ ASAP MS for comparison to prior GC-MS results. Glass capillaries were used for sample introduction by submerging the tip into the prepared methanolic solution. The capillary was then inserted into the probe inlet, where the sample was volatized at 450°C. This method of introduction, referred to as a "burn", was performed a minimum of three times per sample over a 3-minute analytical run time.

Results: When compared to the original GC-MS data, the most abundant analyte was successfully identified in all samples (Table 1). However, in several instances, lower-abundance analytes identified by GC-MS were not detected by the RADIAN™ ASAP-MS system and were present at levels as low as 0.2% relative to the dominant compound. In some cases, additional dilution was required due to detector saturation. For the single liquid sample analyzed, no detectable peaks were observed by the RADIAN™ ASAP-MS, indicating that the sample did not adequately volatize under the analytical conditions, and therefore, did not produce successful "burns."

Item #	Sample Type	GC-MS Results	RADIAN ASAP-MS Results
1	Crystal rocks	Methamphetamine*	Methamphetamine, Amphetamine
2	Pink Tablet	4-Bromo-2,5-Dimethoxyphenethylamine*(2C-B)	2C-B
3	Orange Tablet	MDMA*, MDA*, Cocaine, Ketamine	MDMA, MDA
4	White Pill	Bromazolam*	Bromazolam
5	Blue/Purple Powder	Morphine, 6-Monoacetylmorphine(6-MAM), Fentanyl*, Papaverine	6-MAM, Fentanyl
6	Clear Liquid	Pentobarbital*	No Drugs Identified
7	White Pill	Acetaminophen*, N,N-Dimethylpentylone, Xylazine, Protonitazene	Acetaminophen, Protonitazene, Isotonitazene
8	Pink Powder	MDMA*, Ketamine*, Oxycodone	MDMA, Ketamine
9	White Pill	Bromazolam, Protonitazene*	Protonitazene, Isotonitazene
10	Makeshift Cigarette	MDMB-4en-PINACA*, ADB-BUTINACA	MDMB-4en-PINACA

Table 1. Comparison of analytes identified by GC-MS and RADIAN™ ASAP-MS. *Refers to the dominant compound detected by GC-MS abundances.

Discussion: The RADIAN™ ASAP-MS system demonstrates utility as a rapid screening tool for identifying predominant analytes in drug evidence. Limitations include detector saturation and reduced sensitivity for compounds with varying volatility, which may be improved with dilutions and temperature programming. While GC-MS remains more sensitive for complex mixtures, the speed of the RADIAN™ ASAP-MS system provides practical value for a postmortem toxicology laboratory.

Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

P-206

From Method to Matrix: Analytical Method Development and Pre-Validation Recovery Optimization of Neurotransmitters for Bioanalysis on LC-QTOF-MS

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Abstract

Introduction: Neurotransmitters are endogenous chemical messengers that transmit information across neurons. They play essential roles in the function of the nervous system, such as motor coordination, cognition, and mood. Their dysregulation is believed to be responsible for the pathogenesis of central nervous system (CNS) disorders. Additionally, external factors, such as drug exposure, can cause an imbalance in their levels, resulting in downstream behavioral effects. Neurotransmitters have been assessed as potential biomarkers for neurological conditions. Quantitative neurotransmitter analysis provides supporting evidence for their imbalance in CNS disorders and could inform on targeted therapies. The development of a sensitive and selective method is critical to accurately measuring these neurochemicals that exist in low concentrations in biological matrices.

Objectives: To develop liquid chromatography-quadrupole-time-of-flight mass spectrometry (LC-QTOF-MS) methods to measure 13 neurotransmitters and metabolites and assess their extraction recovery in rat plasma.

Methods: Dopamine, serotonin, acetylcholine, gamma-hydroxybutyric acid (GHB), gamma-aminobutyric acid (GABA), glutamate, homovanillic acid (HVA), 3,4-dihydroxyphenylacetic acid (DOPAC), 5-hydroxyindole-3-acetic acid (5-HIAA), choline, 3,4-dihydroxybutyric acid (DHB), succinic acid, glutathione (GSH), and their internal standards were tuned on a Sciex X500R QTOF. On a Shimadzu Nexera LC 40, a systematic approach was used to evaluate three HILIC columns for mobile phase composition and pH across various gradient methods, assessing chromatographic performance.

To evaluate extraction recovery, 1:100 diluted rat plasma, blank and spiked with 1000 ng/mL of analytes, was extracted using ice-cold 50:50 MeOH + 0.5% FA:H_2O. The supernatant was injected directly or further diluted with organic solvent at a neutral, acidic, or basic pH to assess the most optimal final solution. Pre-spiked and post-spiked samples were analyzed to calculate the extraction recovery.

Results: Acetylcholine, dopamine, serotonin, GABA, glutamate, 5-HIAA, choline, and GSH were tuned in positive mode and chromatographically separated on a Macherey-Nagel NUCLEODUR HILIC column (100 x 2 mm, 3 µm). GHB, HVA, DOPAC, DHB, and succinic acid were tuned in negative mode and combined on another Macherey-Nagel NUCLEODUR HILIC column (50 x 2 mm, 3 µm).

The extraction recovery from the supernatant alone was compared to supernatant diluted with neutral, acidic, or basic ACN (based on MS ionization mode) or 75:25 MeOH:ACN (for negative mode). The supernatant alone resulted in recoveries ranging from 37-142% (4-30 %CV), but significant peak splitting was observed for some analytes in positive mode. The neutral ACN

dilution resulted in better peak shape and recoveries ranging from 77-113% (1-22 %CV), except for 5-HIAA at 135% (16 %CV) and GSH at 186% (8 %CV). The dilution with acidic or basic ACN resulted in recoveries exceeding 100% for 6 analytes. The 75:25 MeOH:ACN dilution resulted in recoveries ranging from 74-94% (9-40 %CV). Due to the high recovery and low %CV, the dilution with neutral ACN was selected for the final method.

Discussion: Two methods were successfully developed to measure 13 neurochemicals with their internal standards. With the additional 1:5 ACN dilution, an extraction recovery of 77–186% across the analytes was achieved. With future pre-validation stability studies and full validation, these methods could be used to measure multiple neurotransmitters in biological matrices.

Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

P-207

Beyond the Usual Suspects: Comprehensive Cannabinoid Screening in Whole Blood

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Abstract

Introduction: According to the Substance Abuse and Mental Health Services Administration, an estimated 64.2 million people 12 or older in the U.S. used cannabis in 2024. Reclassification of marijuana to Schedule III by the federal government could result in an increase in its use medically and recreationally. There are limited regulations in many states regarding medical and/or recreational cannabis products and/or delta-9-tetrahydrocannabinol (Δ9-THC). The recreational market has taken advantage of this by producing synthetic and semi-synthetic cannabinoids to circumvent current regulations. New analytical methods are needed for many of these synthetic and semi-synthetic cannabinoids.

Objectives: To develop and validate a method to detect 21 phyto, synthetic, and semi-synthetic cannabinoids in whole blood using an Agilent 1290/6470 Infinity II liquid chromatography–tandem mass spectrometer with Electrospray Ionization (LC-MS/MS ESI). Samples received by the District of Columbia's Office of the Chief Medical Examiner (DC OCME) were tested to assess the prevalence of cannabinoids.

Methods: Chromatographic separation was achieved using an Agilent LC-MS/MS ESI 1290/6470 Infinity II, a Poroshell 120 EC-C18 Column (3.0 mm x 50 mm x 2.7 mm) and a Poroshell 120 EC-C18 Guard Column (3.0 mm x 5 mm x 2.7 mm) held at 40 °C. Mobile phase consisted of 0.1% Formic Acid in water (A) and 0.1% Formic Acid in Methanol (B). The starting mobile phase ratio was 22:78 (A:B) for 14.50 min, followed by 5:95 (A:B) ratio for 1 minute with a 0.3 mL/min flow rate. The following analytes were analyzed: Δ9-THC, Δ8-THC, 11-nor-9-Carboxy-Δ9-THC (11-nor-9-COOH-Δ9-THC) 11-Hydroxy-Δ9-THC (11-OH-Δ9-THC), 11-nor-9-COOH-Δ8-THC, 9R-Hexahydrocannabinol (9R-HHC), 9S-HHC, (6aR,9R)- Δ10-THC, (6aR,9S)- Δ10-THC, Cannabidiol (CBD), Cannabinol (CBN), Cannabigerol (CBG), Cannabichromene (CBC), MDMB-4en-PINACA, MDMB-4en-PINACA butanoic acid metabolite, MDMB-4en-PICA butanoic acid metabolite, MDMB-4en-BUTINACA, MDMB-4en-BUTINACA butanoic acid metabolite, (S)-5 fluoro ADB, 5-fluoro ADB metabolite 2, 5-fluoro ADB metabolite 7. Samples were extracted using a liquid-liquid method consisting of 90:10 Hexanes: Ethyl Acetate. A calibration curve from 0.25 ng/mL to 100 ng/mL was used to quantitate the analytes. The method was validated to the quantitative analysis standards of ASB 036 Standard Practices for Method Validation in Forensic Toxicology.

Results: The method successfully resolves and quantifies 19 cannabinoids in blood. (6aR,9R)-Δ10-THC and (6aR,9S)-Δ10-THC were only qualitatively identified in blood. The synthetic cannabinoids were all detectable and quantifiable at 0.25 ng/mL. CBC and CBN had a limit of detection (LOD) at 0.5 ng/mL and a limit of quantitation (LOQ) at 1 ng/mL. Δ9-THC, 11-OH-Δ9-THC and 9(S)-HHC had an LOD of 1 ng/mL and an LOQ of 2.5 ng/mL. Δ8-THC, 9(R)-HHC, and CBD had an LOD of 2.5 ng/mL and an LOQ of 5 ng/mL.

Discussion: The American National Standards Board and Academy Standards Board recommend blood confirmation values for Δ9-THC, COOH-Δ9-THC, and 11-OH-Δ9-THC at 1, 5, and 1 ng/mL, respectively. The LOD and LOQ values are set to reflect these recommendations and to maintain relevance to cannabinoid values in the human body. Testing for a range of cannabinoids quickly and cost-effectively is important for laboratories to monitor use and help inform lawmakers and public safety officials.

Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

P-208

Conventional Plasma vs. Dried Microsampled Plasma – Development and Validation of Two Analytical Methods for Adherence Monitoring of Cardiovascular Disease Drugs

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Abstract

Introduction: Plasma analysis has already been established for adherence monitoring. Microsampling offers an alternative sampling strategy for this purpose with potential for self-sampling. The Capitainer-SEP10 device is expected to allow the collection of defined volumes of dried microsampled plasma (DMP) from capillary whole blood suitable for future adherence assessment.

Objectives: The aim of this study was to develop and validate two analytical workflows for adherence monitoring of 15 cardiovascular diseases (CVD) drugs in plasma and DMP, respectively. Their analytical performance should be evaluated and compared.

Methods: Amlodipine, atenolol, atorvastatin, bisoprolol, carvedilol, clopidogrel, diltiazem, lercanidipine, metoprolol, nebivolol, prasugrel metabolite R-138727, rosuvastatin, salicylic acid, simvastatin hydroxy acid, and verapamil were included. For plasma extraction, 300 µL of acetonitrile (containing 0.1% formic acid, *v/v*) and 20 µL of internal standard (IS) solution were added to 50 µL of plasma. For Capitainer-SEP10 samples, 90 µL of water and 10 µL of IS solution were added to one disk (containing 10 µL DMP) for prehydration, followed by 190 µL of methanol for extraction. After shaking and centrifugation, the supernatant was partially evaporated to approximately 80 µL. 5 µL (plasma) or 10 µL (DMP) of supernatant were injected onto a ThermoFisher (TF) TSQ Altis Plus LC-MS/MS system. Chromatographic separation was performed on a C18 column (TF Hypersil GOLD, 100 mm x 2.1 mm x 1.9 µm) using gradient elution with water/acetonitrile. The relative response factors (RRF), determined with a corresponding or structurally related deuterated IS, were used for quantification and the validation using spiked plasma samples was based on recommendations of the European Medicines Agency and the International Association of Therapeutic Drug Monitoring and Clinical Toxicology.

Results: Chromatographic separation of all analytes was achieved within 21 minutes. Lower limits of quantification ranged from 0.2 to 30 ng/mL, except for salicylic acid (100 ng/mL). Isotope dilution mass spectrometry allowed the quantification of all analytes using matrix- and extraction-dependent RRFs, except for prasugrel metabolite R-138727 due to stability issues in the extracted samples. The analytical method was successfully validated including accuracy and precision data for both sampling strategies, and all analytes met the requirements of international guidelines, with exception of lercanidipine, and verapamil (only plasma) and atorvastatin, salicylic acid, and simvastatin hydroxy acid (only DMP). Matrix effects of most analytes were found to be higher for DMP compared to plasma.

Discussion: Two workflows for the analysis of 14 drugs prescribed for treatment of CVD were developed, validated, and compared. Both strategies were found to be suitable for sample collection and extraction. As no external calibration was required, they were shown to be time-saving and sustainable. The accuracy and precision data led to different final quantification ranges for plasma and DMP. The differences in matrix effects between the two sampling strategies could be partially offset by standardization against the corresponding internal standard, as these differences were also observed for the deuterated internal standards. Although the analytical validation showed satisfying results, clinical applicability needs to be demonstrated.

Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

P-209

Enantioselective Analysis of Amphetamines in Serum, Urine, Hair, and Dried Blood Spots

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Abstract

Introduction: Amphetamine and amphetamine-type stimulants, such as methamphetamine and MDMA, rank among the most commonly consumed illicit drugs worldwide. While methamphetamine and MDMA are primarily used for their stimulant effects, amphetamine or its prodrugs can also be prescribed as a medication for attention-deficit hyperactivity disorder (ADHD). Therapeutic drugs most often contain (*S*)-amphetamine as the active compound, whereas illicit amphetamine is typically traded in the racemic form. Chiral analysis of amphetamine enantiomers enables differentiation between therapeutic use of enantiomerically pure amphetamine and additional use of illicit amphetamine.

Objectives: The aim was to develop and validate a sensitive liquid chromatography-tandem mass spectrometry (LC-MS/MS) method for chiral separation and quantification of amphetamine enantiomers in serum, urine, hair and dried blood spot (DBS) samples.

Methods:A liquid chromatography-tandem mass spectrometry (LC-MS/MS) method was developed and validated for the chiral separation and quantification for seven amphetamine type stimulants: (*S*)-/(*R*)-amphetamine, (*S*)-/(*R*)-methamphetamine, (*S*)-/(*R*)-MDMA, (*S*)-/(*R*)-MDA, (*S*)-/(*R*)-MDEA, (1*S*,2*S*)-/(1*R*/2*R*)-pseudoephedrine and (1*S*,2*R*)-/(1*R*,2*S*)-ephedrine. Chiral separation of the enantiomers was achieved by derivatization with N-(2,4-dinitro-5-fluorophenyl)-L-valinamide to yield diastereomers of the amphetamine type stimulants. Diastereomer separation was performed on a Raptor FluoroPhenyl® column (50 × 2.1 mm, 2.7 µm) using H_2O and acetonitrile (0.1% formic acid, 2mM ammonium formate) as mobile phases. Validation parameters - selectivity, linearity, accuracy, stability, limit of detection (LOD), limit of quantification (LOQ), recovery and matrix effects - were assessed according to the guidelines of the German *Society of Toxicological and Forensic Chemistr*y (GTFCh).

Results: A sensitive enantioselective method with a run time of 9 minutes was successfully developed and validated for all matrices. LODs in serum ranged from 0.18-1.78 ng/mL and LOQs from 0.22-4.32 ng/mL. In urine, LODs between 0.17-2.38 ng/mL and LOQs between 0.03-4.73 ng/mL were determined. DBS samples exhibited LODs ranging from 0.89-1.96 ng/mL and LOQs ranging from 2.26-4.97 ng/mL, while LODs and LOQs in hair samples ranged between 0.53-7.25 pg/mg and 2.48-24.06 pg/mg. Linearity ranges of the different analytes were between 1-100 ng/mL and 5-100 ng/mL in serum and urine, 5-150 ng/mL in DBS and 10-1,000 pg/mg in hair. All validation parameters met the requirements of the GTFCh guideline including ranges of accuracy and precision within ± 15-20% and matrix effect data within ± 25%. The method was successfully used for quantification of amphetamine enantiomers in 10 routine samples as well as 5 proficiency tests.

Discussion: The method enables retrospective monitoring of therapy compliance in ADHD patients, as well as differentiation between therapeutic administration of (*S*)-amphetamine and abuse of illicit street amphetamine. Additionally, the inclusion of DBS samples offers a less invasive sampling process. Due to the smaller sample volume of DBS (20 µL) compared to serum (100 µL), this matrix exhibits a higher linearity range at the price of higher LODs and LOQs. One of the main future applications of this method will be studies on the enantioselective metabolism of these substances in the various matrices.

Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

P-210

Performance Evaluation of a Multi-Analyte LC–MS/MS Method in Blood Over Four Years of Application in Routine Casework

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Abstract

Introduction: The installation of a new liquid chromatography–tandem mass spectrometry (LC–MS/MS) system, enabled the implementation of an in-house developed and validated, in accordance with international guidelines, method [Florou et al. J.C.B 2024] for the simultaneous screening and quantification of over 100 analytes in whole blood since mid-2022.

Objectives: This study evaluates the long-term performance of this multi-analyte LC–MS/MS method during routine application in clinical and forensic casework.

Methods: More than 5,000 samples comprising clinical (e.g. overdoses), forensic (e.g. DUI, child neglect), and autopsy cases were analyzed from mid-2022 to mid-2026. The analytical procedure employed a single-step liquid–liquid extraction of whole blood with acidified MTBE and multiple reaction monitoring (MRM) acquisition of mass spectra. The target analytes included drugs of abuse (DOA), prescription and over-the-counter drugs commonly involved in poisoning and medical malpractice cases in our territory, as well as new psychoactive substances (NPS) and toxic substances associated with adverse effects. Limits of detection (LOD) and quantification (LOQ) values were 0.01–5 ng/mL and 0.05–20 ng/mL, respectively, linearity between 0.05- 500 ng/mL ($R^2 \geq 0.9811$), inter- and intra-day precision ranged between 3–15% and 7–18%, respectively, accuracy was within acceptable limits, matrix effect was within ±25%, stability exceeded 85%, and no carryover was observed [Florou et al. J.C.B. 2024]. Quality control (QC) samples were analyzed across batches to test accuracy and robustness over time. The results were evaluated in respect to case types and drug classes.

Results: Most of the samples analyzed (60%) were from autopsies, following by forensic (drug-facilitated crimes, DOA verification, child neglect investigations etc.) and clinical (pediatric cases, drug intoxications, suicide attempts) cases. The most frequently detected drug classes in autopsy samples were antidepressants (28%), analgesics (26%), and benzodiazepines (21%), followed by anesthetics (9%), classic drugs of abuse (7%), antipsychotics (4%), and other substances (5%). Moreover, 85% of cases were positive for at least one drug while more than one substance was detected in the 90% of positive cases. Major classes for forensic cases were DOA and benzodiazepines. Overall, 70 out of 100 substances were detected in toxic, therapeutic and sub-therapeutic concentrations. The analytical protocol proved effective for both screening and quantitative analysis, of different blood types (whole, serum, and plasma), from antemortem and postmortem cases, even putrefied samples. Furthermore, the method demonstrated flexibility and proved functional for semi-quantitative analysis of 19 additional analytes depending on the analytic needs and specific characteristics of each case. Finally, the chromatographic and mass-spectroscopic settings were applied to analyze extracts of other biological matrices such as urine, hair, and tissues. The analysis of QC samples revealed no significant limitations affecting routine application.

Discussion: Over the four-year period of routine application, the method has demonstrated exceptional analytical reliability and operational robustness, making it a fit-for-purpose approach for comprehensive toxicological screening and quantification in routine casework. A major strength is its flexibility is the addition of new analytes to address emerging drugs and NPS, broadening further the spectrum of analytes on demand of routine and emergent toxicological casework.

Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

P-211

Analytical Challenges in Forensic Chemistry Identification of Emerging Peptide and Protein Substances in Seized Materials

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Abstract

Introduction: The availability of peptide- and protein-based substances marketed for performance enhancement as well as aesthetic, metabolic, and wellness-related purposes has increased substantially in recent years. Often advertised on online platforms and distributed through non-regulated supply chains, these substances present a growing challenge for forensic chemistry laboratories tasked with identifying seized materials submitted by customs and law enforcement authorities. Seized substances include classical doping-related substances such as growth hormone analogues and growth hormone-releasing peptides but also synthetic peptides with variable purported effects such as weight-loss and anti-inflammation.

Objectives: This study presents analytical strategies and challenges associated with the forensic identification of peptide- and protein-based substances in seized materials, with emphasis on structural diversity, limited reference data, and the applicability of LC-MS/MS based workflows. Furthermore, recent findings from the analysis of seized material will be presented.

Methods: Seized materials submitted by customs authorities were analyzed using liquid chromatography–mass spectrometry (LC-MS) platforms. Top-down (intact protein) and bottom-up (proteolytic cleavage) proteomics approaches were employed for analysis of target proteins. High-resolution LC-MS/MS (quadrupole Time-of-Flight) detection was applied and optimized for high-mass and multiple-charged molecules using data-dependent acquisition (DDA). Analytical workflows included targeted analysis with reference standards and structural elucidation based on MS/MS fragmentation data.

Results: Human growth hormone (recombinant HGH 22 kDa) was successfully identified as a full-length protein, characterized by multiple-charged ions (dominant ion $[M+16H]^{16+}$) and confirmed via identification of 4 different proteotypic peptides generated by trypsin cleavage.

Analysis of recent seizures revealed a diverse portfolio of synthetic peptides, exemplified by retatrutide (a triple agonist weight-loss candidate), BPC-157 (Body Protective Compound with regenerative and cytoprotective effects), MOTS-c mitochondrial peptide (metabolism regulation), copper-GHK (a skin-remodeling peptide), Ipamorelin (growth hormone release).

In a representative case involving 18 vials from a single batch, significant substance heterogeneity and inconsistent labeling were observed. While nine compounds could be tentatively identified using LC-MS-based approaches targeting peptide-like species, several species exhibited clear peptide-like characteristics but did not match existing reference data library. Ongoing research will attempt identification from MS/MS data using e.g. de novo peptide sequence analysis.

Discussion: The substances identified in this study range from short peptides, larger synthetic peptides often containing non-natural amino acids and chemical modifications, to full-length recombinantly produced proteins. Poorly labeled products, limited availability of library data, and the difficulties in *de novo* peptide sequence identification further underscore the significant challenges in forensic peptide analysis. The analysis of peptide-related substances may constitute an expanding category within future forensic chemistry, requiring continued methodological development and awareness.

Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

P-212

Simultaneous LC-MS/MS Determination of Anabolic Androgenic Steroids and Selective Estrogen Receptor Modulators in Postmortem Blood: Validation and Forensic Application

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Abstract

Introduction: Misuse of anabolic androgenic steroids (AAS) remains a persistent issue in forensic toxicology, particularly among bodybuilders and users of performance-enhancing drugs. Selective estrogen receptor modulators (SERMs) are often co-administered with AAS to reduce estrogen-related adverse effects during steroid cycles. Although analytical methods for AAS or SERMs individually have been reported, validated methods enabling simultaneous determination of both classes in postmortem blood remain limited.

Objectives: To develop and validate an LC-MS/MS method for simultaneous determination of 19 AAS and 10 SERMs in postmortem blood and to evaluate its forensic applicability using authentic case samples.

Methods: Postmortem blood samples were prepared by protein precipitation using methanol, followed by evaporation and reconstitution prior to LC-MS/MS analysis. Chromatographic separation was achieved on a C18 column using gradient elution with a total runtime of 17.5 min. The method was validated in accordance with the SWGTOX and ANSI/ASB guidelines for forensic toxicology method validation. Detection was performed by tandem mass spectrometry in multiple reaction monitoring mode. Validation parameters included selectivity, linearity, limit of detection (LOD), limit of quantification (LOQ), intra- and inter-day precision, accuracy, recovery, and matrix effect.

Results: Selectivity was confirmed using six blank postmortem blood samples, with no significant interfering peaks observed. Calibration curves were linear over 1–500 ng/mL for AAS and 25–500 ng/mL for SERMs ($R^2 \geq 0.99$). Intra- and inter-day precision were below 13.8%, and bias ranged from –14.4% to +15.0%. Recoveries were 69.3–129.4%, while matrix effects ranged from 70.5–132.8%. Application to three bodybuilding-related postmortem cases confirmed the forensic utility of the method. In Case 1, nandrolone decanoate was identified in heart blood (HB) (29.9 ng/mL) and peripheral blood (PB) (20.2 ng/mL), along with tamoxifen in HB (30.1 ng/mL) and PB (29.8 ng/mL). In Case 2, boldenone was detected in HB (23.3 ng/mL), and testosterone was found in HB (6.7 ng/mL) and PB (26.1 ng/mL), while epitestosterone was below the LOQ in both matrices. In Case 3, testosterone was observed in HB (2.5 ng/mL), PB (14.4 ng/mL), and urine (56.1 ng/mL), whereas boldenone was detected only in urine (28.2 ng/mL), and urinary epitestosterone was measured at 9.0 ng/mL

Discussion: The validated LC-MS/MS method enables the simultaneous detection of AAS and SERMs in postmortem specimens and reflects current co-use patterns associated with bodybuilding-related drug abuse. Inclusion of both drug classes in a single assay improves analytical efficiency, broadens toxicological coverage, and may reduce overlooked findings when only one class is targeted. This method is expected to be applicable to routine forensic casework, postmortem investigations, and surveillance of emerging performance-enhancing drug misuse.

Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

P-214

Rapid Workflows for Triple Quadrupole LC-MS/MS Screening

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Abstract

Introduction: The rapid emergence of novel psychoactive substances has reshaped the drug landscape and placed new demands on toxicology testing. Traditional immunoassay-based screens, while long used as a first line approach, cannot keep pace with shifting drug trends because assay development is slow. In contrast, MS-based screening assays can be adapted quickly to incorporate new analytes, making them better suited for the dynamic environment. As toxicology laboratories face increasing workloads, screening procedures are essential for limiting the number of specimens requiring resource intensive confirmatory testing. An effective screening method must be fast, inexpensive, and simple to execute while confidently excluding most negative samples without increasing the risk of false negatives.

Objectives: To meet these emerging challenges, a rapid LC-MS/MS screening method was developed to enable flexible implementation of multiple workflows based on matrix, sample preparation approach, analyte panel scope, and confidence requirements. The method was intentionally designed to integrate with the Shimadzu Forensic Toxicology Database, providing a scalable platform that can be readily adapted to evolving drug landscapes.

Methods: Fifty drugs of abuse, including isobars such as morphine and hydromorphone as well as codeine and hydrocodone, were separated on a Shim-pack Scepter PFPP-120 column (1.9 µm, 2.1x50 mm) with 2 mM ammonium formate and 0.002% formic acid as additives in water (mobile phase A) and methanol (mobile phase B) utilizing gradient elution. The gradient ramps from 55-100% B over 2.5 minutes. The compounds were analyzed by positive and negative mode electrospray ionization (ESI). Three screening acquisition methods were compared: MRM, MRM Spectrum Mode, and triggered Product Ion Scans.

Results: The LC-MS/MS method achieved complete chromatographic separation of isobaric compounds, enabling identification of all targeted analytes across workflows. Consistent performance was observed for each method configuration.

Calibration curves and limits of detection are presented for all three methods, allowing informed evaluation of relative sensitivity and selection of an appropriate screening workflow.

Discussion: This work demonstrates rapid (5 min total runtime) triple quadrupole LC-MS/MS screening for toxicology applications. Complete chromatographic separation of isobaric compounds was achieved.

The method was designed for direct integration with the Shimadzu Forensic Toxicology Database, which currently includes more than 2,500 compounds, enabling efficient panel expansion and long-term adaptability as drug trends evolve. In addition, the library editor features of Insight Screening allow new compounds to be incorporated as reference spectra are published by the Center for Forensic Science Research & Education (CFSRE), supporting early detection and triage of emerging substances that may warrant external confirmatory testing.

While optimized for triple quadrupole instrumentation, the workflow concepts may also be applied using single quadrupole mass spectrometry when laboratories are willing to accept the possibility of a slightly higher percentage of samples being referred for confirmatory testing. This flexibility illustrates how screening strategies can be tailored to available instrumentation and preferred analytical framework.

Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

P-215

Development of Monoclonal Antibodies Targeting Kratom Alkaloids and Nitazene Opioids: From Hapten Design to Immunochemical Applications

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Abstract

Introduction: The rapid emergence of new psychoactive substances, including kratom alkaloids and highly potent synthetic opioids of the nitazene class, presents a significant analytical and forensic challenge. Current detection approaches rely predominantly on advanced instrumental techniques; however, there is a critical need for rapid, sensitive, and field-deployable screening tools. Immunochemical methods, particularly those based on monoclonal antibodies, offer a promising solution, provided that high-affinity and selective antibodies against small-molecule targets can be generated.

Objectives: This study aimed to (i) design and synthesize hapten derivatives of selected kratom alkaloids (primarily mitragynine) and representative nitazenes, (ii) prepare immunogens and induce immune responses in rabbits, (iii) develop and characterize monoclonal antibodies, and (iv) evaluate their applicability in immunochemical formats (ELISA, LFIA) for rapid detection of these compounds.

Methods: Haptens were synthesized via structural modification of target molecules to introduce functional groups suitable for conjugation while preserving key antigenic determinants. Immunogens were prepared through covalent coupling of haptens to carrier proteins via activated carboxyl groups. Rabbits were immunized using established protocols for small-molecule immunogens. Resulting sera were processed, and monoclonal antibodies were generated using recombinant expression systems. Antibody binding affinity was evaluated using competitive immunoassays. The equilibrium dissociation constant (Kd) between the monoclonal antibody and the small-molecule drug was determined using competitive assay principles. Results are reported as mean ± SD, calculated from measurements performed using at least four different analyte concentrations. Selected antibodies were further characterized for cross-reactivity against structurally related alkaloids and metabolites (mitragynine, 7-hydroxymitragynine and mitraphylline) and against panels of nitazene analogues.

Results: The developed monoclonal antibodies demonstrated ultra-high affinity towards target analytes, with Kd values in the low nanomolar to sub-nanomolar range for mitragynine (e.g., ~0.10–0.95 nM depending on clone). Importantly, significant reduction in affinity was observed for structurally related but non-target alkaloids, indicating favorable selectivity profiles. For nitazenes, antibody panels exhibited broad recognition across multiple derivatives, including etonitazene, isotonitazene, and related desnitazenes, enabling group-specific detection of this structurally diverse class. The antibodies were successfully implemented in ELISA and lateral flow assay formats, demonstrating their suitability for rapid screening applications.

Discussion: The results confirm that rational hapten design enables the generation of high-affinity monoclonal antibodies against structurally complex small molecules such as kratom alkaloids and nitazenes. The observed affinity and selectivity profiles are suitable for both laboratory-based immunoassays and field-deployable detection systems. The broad reactivity of anti-nitazene antibodies represents a significant advantage for forensic applications, where unknown or emerging analogues frequently occur. Conversely, selective recognition of mitragynine over related alkaloids supports targeted detection of kratom use. These findings provide a robust foundation for the development of rapid immunochemical screening tools, addressing an urgent need in forensic toxicology and public health surveillance. This study demonstrates a complete workflow from hapten synthesis to functional monoclonal antibodies capable of detecting emerging opioid threats and kratom alkaloids. The developed immunoreagents represent a viable platform for next-generation rapid diagnostic assays.

Disclosure

Yes, I, or a member of my immediate family, has a financial interest to disclose.

Conflict of Interest

The study was a join project with the company DIANA Biotechnologies, a.s. and was exclusively supported by the project TREND FW12010013 (Technology Agency of the Czech Republic)

P-216

Are You Stable? A Working Stock Stability Study

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Abstract

Introduction: Numerous studies have examined the stability of THC and metabolites in blood and in commercially prepared standards, but limited research addresses the stability of in-house prepared standards. Many laboratories assign a one-year expiration date and store these standards under repeated freeze–thaw conditions.

At the Vermont Forensic Laboratory (VFL), this approach was applied to the THC working stocks. During proficiency testing, results trended towards the lower end of acceptable ranges. A quality investigation suggested long-term storage of working stocks might affect overall quantification.

Objectives: This study evaluated whether a one-year expiration period for THC working stocks is appropriate and whether single-use aliquots improve analytical accuracy.

Methods: The THC panel includes delta-9-tetrahydrocannabinol (THC), 11-hydroxy-tetrahydrocannabinol (THC-OH), and 11-nor-9-carboxy-tetrahydrocannabinol (THC-COOH).

Calibrators and internal standards (ISTD) were obtained from one manufacturer, while quality control (QC) standards were sourced from another. Working stocks were prepared in methanol at the following concentrations:

	THC	THC-OH	THC-COOH
Calibrator	1 ug/mL	1 ug/mL	10 ug/mL
QC	1 ug/mL	1 ug/mL	10 ug/mL
ISTD	500 ng/mL	500 ng/mL	5 ug/mL

Calibrator and QC stocks were divided into single-use aliquots and stored in a freezer, while ISTD stocks were stored in bottles with minimal headspace in a freezer.

Calibrators were prepared in ranges of 0.5–50 ng/mL (THC, THC-OH) and 5–500 ng/mL (THC-COOH). QC samples were prepared at 1.5, 25, and 40 ng/mL (THC, THC-OH) and 15, 250, and 400 ng/mL (THC-COOH).

Samples were extracted using 500 µL of negative human blood, 25 µL of ISTD, and 25 µL of working stocks. A fortified matrix pool (FM) was also extracted to mimic routine casework analysis. Samples were extracted using solid phase extraction (SPE) and analyzed on a Waters Xevo TQS-Micro LC-MS/MS system using a C18 BEH column.

Results: Percent bias comparisons were calculated monthly and compared to the initial analysis, focusing on the area ratio of the highest calibrator and the quantification of the high QC and FM results. A ±20% acceptance criterion was applied.

Single-use QCs showed a gradual negative bias over time, reaching approximately -20% by month 11. In contrast, our original QC storage method, which induces freeze-thaw cycles, demonstrated a smaller change of bias for 6 months, with a maximum bias of -10%.

For the calibrator, both single-use and freeze-thaw demonstrated similar bias changes within their respective timeframes, observing a maximum bias of -17.5% for single-use and -16.5% for freeze-thaw.

The FM bias reached -20% by month 10 using the single-use calibrator.

Discussion: F-test analysis supported that working stocks remained stable for approximately 10 months. However, comparison with 6 months of previous casework suggested that switching to single-use aliquots had minimal impact on overall accuracy.

Several limitations should be considered. Triplicate analyses used during initial runs may have inflated day 0 results, affecting bias calculations. Additionally, the study design did not directly compare single-use aliquots and freeze-thaw in parallel, limiting the strength of conclusions.

Given these factors, VFL adopted a conservative approach, implementing single-use aliquots with a 9-month shelf life to minimize potential degradation and to maintain confidence in analytical results.

Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

P-217

Metabolite Masquerade: False-Positive Norfentanyl in the Wake of Local Anesthetics

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Abstract

Introduction: Liquid chromatography-tandem mass spectrometry (LC-MS/MS) is the gold standard for the quantification of fentanyl and norfentanyl (NF), a metabolite formed via N-dealkylation at the piperidine ring. With the rise of illicitly manufactured fentanyl (IMF), local anesthetics (LAs) such as bupivacaine, mepivacaine, and ropivacaine are increasingly encountered as adulterants in postmortem toxicology with effects ranging from agitation, anxiety, dizziness, numbness of limbs, racing heart, to slowed breathing. These compounds undergo N-dealkylation of the piperidine ring to form 2',6'-pipecoloxylidide (PPX), sharing the same molecular weight (m/z 232) and chemical formula (C14H20N2O) as NF. This structural similarity presents a potential source of analytical interference and misidentification.

Objectives: To investigate potential interferences from LAs (bupivacaine, mepivacaine, and ropivacaine) with NF detection, we evaluated their metabolism and fragmentation using LC-QTOF-MS and LC-MS/MS to understand their role in producing false positive results for NF.

Methods: Postmortem femoral blood specimens were collected at autopsy at the Travis County Medical Examiner (TCME) in Austin, TX and antemortem specimens were collected at the emergency department. Qualitative screening was performed using GC-MS in full scan mode (prior to 2021) or with a comprehensive LC-QTOF-MS method following acetonitrile protein precipitation. The LC-QTOF-MS screens for over 350 drugs with a 1 ng/mL limit of detection for fentanyl and NF. Quantitative analysis of fentanyl and its metabolite (NF) was performed via LC-MS/MS following an alkaline liquid-liquid extraction and formic acid back extraction (LOQ: 0.5 ng/mL). Interference studies were conducted for bupivacaine, mepivacaine, ropivacaine, and PPX at 1000 ng/mL.

Results: Since 2020, at least 10 postmortem cases screened positive for NF, however, confirmatory analysis failed ion ratio criteria for NF (product ions m/z 84 and 55) despite apparent elevated concentrations. Review of these cases identified the presence of LAs (bupivacaine, mepivacaine, or ropivacaine) in all samples. No interferences with NF were observed from all three LAs; however, these compounds metabolize to PPX (via N-dealkylation of the piperidine ring), which shares the same molecular weight and key product ions as NF. Analysis of a PPX reference standard by LC-QTOF-MS confirmed similar fragmentation patterns (m/z 84 and 150), with the absence of m/z 177 distinguishing PPX from NF across the concentration range. PPX eluted ~0.3 min earlier than NF (3.63 min) on the LC-QTOF-MS but was almost identical by LC-MS/MS. PPX produced an interference during LC-MS/MS quantitation at the same transitions as NF. Retrospective data analysis confirmed the presence of PPX in all 10 cases.

Discussion: These findings demonstrate that bupivacaine, mepivacaine, and ropivacaine can produce false positive NF results through their metabolism to PPX. This is particularly concerning given the increasing presence of these LAs as adulterants in IMF in addition to their medicinal use. Inadequate chromatographic resolution and overlapping transitions may lead to the misidentification of NF, especially in laboratories relying on targeted LC-MS/MS methods. Careful evaluation of chromatographic separation and recognition of distinguishing spectral features, such as the absence of m/z 177 in PPX, are critical to reduce misidentification. These data highlight the importance of comprehensive analytical testing and increased awareness of emerging interferences in fentanyl testing.

Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

P-218

Development and Validation of an Analytical Workflow for the Analysis of Psychedelics in Whole Blood

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Abstract

Introduction: Depression affects over 264 million individuals worldwide. Traditional treatment methods are often limited in scope and have significant drawbacks. Since 2018, noteworthy advancements in new age treatments have propagated legislative initiatives and reform regarding psychedelic compounds. Legislative changes including medical applications, decriminalization, and legalization of psychedelic compounds directly impacts forensic toxicology laboratories nationwide. Given the analytical challenges associated with these compounds, forensic toxicology laboratories are left to develop and validate new analytical workflows for the identification and quantitation of psychedelic compounds for driving under the influence of drugs (DUID) and postmortem cases. An analytical workflow for the identification and quantitation of these compounds and their main metabolites needs to consider sample preparation parameters to minimize the implications associated with their inherent instability.

Objectives: The objective of this research project was to develop and validate an analytical workflow for the identification and quantitation of psychedelic compounds in whole blood using liquid chromatography tandem mass spectrometry (LC-MS/MS).

Methods: A method was validated in accordance with ANSI/ASB Standard 036, Standard Practices for Method Validation in Forensic Toxicology for antemortem blood and postmortem blood. An LC-MS/MS method was developed for the qualitative/quantitative analysis of 15 psychedelic compounds and associated metabolites using an Agilent Technologies 1260 Infinity II liquid chromatograph and a 6460 mass spectrometer. A gradient elution with a starting mobile phase of 96%:4% (0.01% formic acid in water:0.01% formic acid in methanol) was employed using a Zorbax Eclipse Plus C18 RR column (2.1 x 100 mm, 3.5 µm). Electrospray ionization in positive mode using Dynamic Multiple Reaction Monitoring (MRM) was used for detection. The total run time was 8.50 minutes allowing for chromatographic separation, identification, and quantitation.

Results: The optimal sample preparation technique for the extraction of psychedelic compounds in biological matrices was a protein precipitation. A total of 1.0 mL of biological matrix was precipitated with 2.0 mL of acetonitrile. Following centrifugation, the supernatant was transferred, dried down under nitrogen (45°C), and reconstituted in 100 µL of 0.01% formic acid in water.

The compounds evaluated include psilocin, psilocybin, N,N-DMT, 5-methoxy-DMT, N,N-DMT-N-oxide, 5-methoxy-DiPT, 4-hydroxy-DiPT, 4-acetoxy-DET, 4-acetoxy-DMT, 4-hydroxy-DET, 5-hydroxy-DMT, 4-hydroxyindole-3-acetic acid, 5-methoxy-NiPT, 2-oxo-3-hydroxy-LSD, and LSD. The validation consisted of bias and precision, estimated limit of detection, lower limit of quantitation, carryover, interferences, dilution integrity, and stability. The working range for quantitative compounds was 1-100ng/mL with the exception of psilocin which has a

working range of 2-200 ng/mL. The best fit calibration model for all compounds was quadratic weighted 1/x with the exception of 5-methoxy-DiPT which was best fit to a quadratic non-weighted model. Significant ionization suppression (>25%) was noted for nearly all compounds in blank blood, antemortem blood, and postmortem blood. This was recognized to be more significant with later eluting compounds.

Discussion: A method was developed for the qualitative and quantitative analysis of 12 psychedelic compounds in antemortem blood and postmortem blood using liquid chromatography-tandem mass spectrometry. The method was validated in each matrix type in accordance with ANSI/ASB Standard 036, Standard Practices for Method Validation in Forensic Toxicology.

Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

P-219

Re-Evaluation of Sample Preparation for Extraction Efficiency Improvement

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Abstract

Introduction: Sample preparation, including calibrator fortification, is a necessary step prior to instrumental analysis. When a single calibrator fails acceptance criteria causing reporting range limitations, reanalysis may be required, which involves additional use of not only sample volume, but also laboratory resources such as solvents, instrument consumables and analysts' time. The observation of analysts often having to exclude points, mostly due to low volume used in fortification, from a calibration curve prompted our laboratory to re-evaluate calibrator and control preparation in blank matrix.

Objectives: To simplify and standardize the steps to dilute calibrators and controls used to fortify blank blood matrix prior to extraction for current quantitative confirmation methods.

Methods: Previously one certified reference material (CRM) was diluted to make three working calibration solutions while a separate CRM was diluted to make two working control solutions. Those respective working solutions were used to fortify a six-point calibration model with three controls. The volume of those working solutions being fortified into blank matrix ranged from 10-50 µL and required the addition of methanol to all samples. The newly validated method requires one calibration stock solution and one control stock solution. During extraction, serial dilutions of the new respective stocks are first prepared with methanol, a consistent volume of 50 µL is taken to achieve target concentrations for each calibrator and control and then fortified with 500 µL of blank matrix to create a seven-point calibration model with three controls. A verification of the new process was performed by three analysts and studies included bias and precision, linearity of calibration model and the analyses of blind samples comparing both the old and the new methods.

Results: Comparing linearity, both methods were similar, with the new preparation showing slight improvement. Combining both processes into one analytical run, the r2 of the previous was 0.9994 versus 0.9998, with the new. Across multiple runs, bias and precision were tested. The bias of the three control levels using the new method of all three runs was 2.76%, -1.05% and -2.71% while the %CV was 2.16%, 3.60% and 2.92%. These are all within the acceptable range of being less than or equal to ±20%.

Spiked samples, unknown to the analysts, were analyzed to test the removal of the addition of methanol to show the method is still able to achieve precise results while reducing a step in the sample preparation. The accuracy of these samples was improved among all three analysts with an average percent difference being -16.57% and 0.15% for the old and new method, respectively.

Discussion: Analytical challenges can arise which can test the strength of validated methods. Regularly reviewing data to optimize a process or workflow can help negate these challenges. A new process of in-extraction dilutions (instead of multiple prepared working solutions) was implemented to help analysts – both new and experienced – improve extraction efficiency by removing low volumes used in fortification and the addition of methanol. This led to reduced number of re-analyses and improved turnaround time. At this time, this process of preparing calibrators and controls has been applied to two confirmation assays and will soon be implemented to the remainder.

Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

P-220

Column Lifetime Performance in LC–MS/MS Pain Management Drug Testing: A Comparative Study

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Abstract

Introduction: High-throughput toxicology laboratories depend on robust LC-MS/MS workflows with minimal downtime and consistent analytical performance to support large, multi-analyte drug panels for urine drug testing. Consistent column performance is an important yet often limiting factor in routine toxicology testing. Complex biological matrices and high injection volumes can lead to rapid column fouling, increased backpressure and degraded peak shape which affects result quality and necessitates more frequent column replacement. This reduces instrument uptime and directly impacting laboratory productivity and increases time to result and cost.

Objectives: To compare column lifetime of standard Kinetex and Kinetex Endrix columns with a proprietary hardware modification to reduce column plugging for an LC-MS/MS urine pain management panel.

Methods: Three Kinetex® Endrix columns with modified hardware designed to reduce column plugging and three standard Kinetex® (conventional column hardware) biphenyl columns, 50 × 3.0 mm, 2.6 µm, 100 Å, were evaluated using a urine toxicology panel with 75 drugs and metabolites including opioids, benzodiazepines, amphetamines, barbiturates, antidepressants, muscle relaxants, and cannabinoids.

LC-MS/MS quantitation was achieved using a SCIEX 5500. Mobile phase A was 0.1% formic acid in water and mobile phase B was 0.1% formic acid in methanol. Urine samples were hydrolyzed and extracted using Beta-Gone pass-through SPE. Columns were conditioned with 50:50 water/acetonitrile for 10 min and equilibrated under initial gradient conditions.

Column lifetime performance was assessed by tracking retention time reproducibility, peak width at half height, signal intensity, and backpressure over consecutive injections.

Results: The Kinetex® Endrix columns provided a 50% average increase in number of injections compared to standard Kinetex® columns, while maintaining acceptable retention time stability and peak widths throughout extended testing. Signal intensity differences between the two column types remained minimal (<5%), indicating comparable initial and sustained sensitivity. Notably, after approximately 800 injections, Endrix columns preserved stable backpressure and chromatographic performance, while standard Kinetex® columns showed a marked rise in backpressure accompanied by peak distortion and declining performance of several marker compounds.

Discussion: The analyte panel included several compounds with chromatographic behavior that are highly sensitive to column integrity. Chromatography of amitriptyline and nortriptyline was interrogated as the primary marker pair. Column lifetime was determined by evaluating the integration accuracy of these closely eluting analyte peaks. These analytes were therefore used as markers for determining column end-of-life.

Column lifetime was evaluated by monitoring retention time reproducibility, peak width at half height, signal intensity, and backpressure across consecutive injections. A progressive increase in system backpressure was observed and correlated strongly with declining chromatographic performance, most notably peak broadening and reduced integration quality for the most sensitive analytes. Both column types demonstrated comparable chromatographic performance, with similar resolution, retention times, peak height, peak area, and MS sensitivity for their respective usable lifetimes.

The updated hardware of Kinetex® Endrix columns produced significantly longer operational lifetime without compromising the quality of chromatography or results.

Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

P-221

Performance Evaluation of the ARK Xylazine Assay on the Siemens Atellica DT 250 Analyzer

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Abstract

Introduction: Xylazine is increasingly detected as an adulterant in illicit drug supplies and is associated with significant public health and forensic concerns. Rapid and reliable screening methods are needed for detection of xylazine in biological specimens.

Objectives: Develop an automated enzyme homogeneous immunoassay for detection of xylazine in human urine with a cutoff concentration of 10 ng/mL. Average concentrations of Xylazine in DUID and postmortem cases are 36 ng/mL and 41 ng/mL. The calibrator concentrations range from 0 ng/mL to 500 ng/mL. Evaluate the analytical performance of the Xylazine Assay when implemented on the Siemens Atellica® DT 250 clinical chemistry analyzer. Compare method comparison values to that of the reference values obtained on the Beckman Coulter AU 480 analyzer.

Methods: Performance studies were conducted at ARK Diagnostics (Fremont, CA) to evaluate precision, analytical recovery, method comparison, high sample dilution, sample carryover, and calibration plus reagent stability. Following CLSI guidelines, precision was assessed using a five-day study design. Analytical recovery was evaluated using drug free urine samples spiked with certified xylazine reference material across the assay range. Method comparison included 110 patient specimens analyzed using both the Atellica DT 250 analyzer and the reference Beckman Coulter AU480 analyzer.

Results: Total precision ranged from 3.6% to 5.1% CV for concentrations above 10 ng/mL. Analytical recovery ranged from 92.8% to 108.1% across concentrations of 6 to 400 ng/mL. Method comparison demonstrated strong qualitative agreement with a sensitivity of 100.0% and specificity of 98.4% relative to the 10 ng/mL cutoff. Dilution studies showed recoveries between 100.6% and 104.5% for samples diluted up to 1:1000. No measurable sample carryover was observed from high concentration samples up to 6000 ng/mL. Calibration stability was maintained for 14 days and on-board reagent stability for 30 days.

Discussion: The performance data indicates that the ARK Xylazine Assay is highly dependable on the Siemens Atellica DT 250 analyzer, maintaining strong agreement with the reference method. These results support implementation of the assay as an automated screening method for xylazine detection in forensic and toxicology laboratories.

Disclosure

Yes, I, or a member of my immediate family, has a financial interest to disclose.

Conflict of Interest

Authors are salaried employees of ARK Diagnostics

P-222

Three Approaches, One Target: A Comparative Study of LC-MS/MS, LC-Q/TOF-MS, and ELISA for PCP, Ketamine, and Selected NPS Analogs

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Abstract

Introduction: The rapid emergence of novel psychoactive substances (NPS), particularly structural analogs of phencyclidine (PCP) and ketamine, continues to challenge forensic toxicology laboratories. Traditional screening methods such as enzyme-linked immunosorbent assays (ELISA) remain widely used due to their low cost, automation capabilities, and minimal sample preparation; however, their reliance on antibody cross-reactivity limits detection of structurally modified analogs. Consequently, laboratories have increasingly adopted platforms such as liquid chromatography–tandem mass spectrometry (LC-MS/MS) and liquid chromatography–quadrupole time-of-flight mass spectrometry (LC-Q/TOF-MS). While LC-MS/MS provides highly sensitive and selective analysis, LC-Q/TOF-MS enables high-resolution accurate mass detection, non-targeted screening, and retrospective data analysis. A direct comparison of these three approaches across multiple matrices is critical to inform method selection and workflow design in modern forensic casework.

Objectives: The purpose of this study was to evaluate and compare the sensitivity, selectivity, and practical applicability of ELISA, LC-MS/MS, and LC-Q/TOF-MS for detecting PCP, ketamine, and selected analogs in blood, urine, and oral fluid.

Methods: Neogen© PCP and ketamine ELISA kits were evaluated for cross-reactivity against a panel of target compounds, including deschloroketamine, deschloro-*N*-ethyl ketamine, 2-fluoro-2-oxo-PCE, fluorexetamine, 3-methyl PCP, and 3-methoxy PCP.

For mass spectrometric analysis, a liquid-liquid extraction was optimized using 0.5 mL of matrix, fortified with internal standards, followed by extraction with 10 mM borate buffer (pH ~9) and *N*-butyl chloride. Extracts were evaporated and reconstituted prior to analysis. Chromatographic separation employed 0.1% formic acid in water (mobile phase A) and 0.1% formic acid in acetonitrile (mobile phase B). Instrument parameters were optimized independently for LC-MS/MS (targeted MRM acquisition) and LC-Q/TOF-MS (all ions acquisition) on Agilent platforms.

Method validation followed ANSI/ASB Standard 036, with quantitative validation performed on LC-MS/MS (blood and oral fluid) and qualitative validation on LC-Q/TOF-MS (blood and oral fluid), with additional qualitative assessment in urine for the LC-MS/MS method.

Results: ELISA demonstrated variable cross-reactivity and failed to reliably detect several structural analogs at standard kit cut-offs.

LC-Q/TOF-MS achieved qualitative detection of all analytes at 0.25 ng/mL in blood and oral fluid, with acceptable matrix effects, carryover, and processed sample stability. However, increased between-day variability, mass accuracy challenges, and ion ratio limitations were observed under all ion acquisition conditions.

LC-MS/MS provided the greatest sensitivity and quantitative performance. Limits of detection were 0.5 ng/mL in urine and 0.1 ng/mL in blood and oral fluid, with a validated calibration range

of 0.5–500 ng/mL. Accuracy, precision, matrix effects, carryover, and dilution integrity met acceptance criteria across matrices, with no significant interferences observed.

Discussion: This study demonstrates that ELISA alone is insufficient for comprehensive detection of emerging PCP and ketamine analogs due to limited cross-reactivity. LC-Q/TOF-MS offers clear advantages for non-targeted screening and identification of novel compounds, supporting its role in NPS surveillance and retrospective analysis. In contrast, LC-MS/MS remains the most reliable platform for sensitive and quantitative confirmation but is inherently limited to targeted analyte panels.

These findings support a complementary, multi-tiered analytical workflow integrating immunoassay screening with both targeted and high-resolution mass spectrometric techniques to enhance detection capabilities in forensic toxicology casework.

Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

P-223

Performance Evaluation of Second-Generation ARK Urine Drug Testing Assays on the Siemens Atellica DT 250 Analyzer

Thomas Houts, Jackie Nguyen-Choi, David Wolfson, Uyen Truong, Ki Chung, Soon Oh, Robert O'Malley, Rajendra Singh

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Abstract

Introduction: Enhancements to immunoassays for fentanyl, ethyl glucuronide and ketamine yield superior sensitivity, specificity and help to improve product attributes plus reagent consistency. Second-generation homogeneous enzyme immunoassays are designed to enhance analytical performance, reagent consistency, and overall robustness in high throughput urine drug screening.

Objectives: Product improvement of three ARK Diagnostics homogeneous immunoassays for Fentanyl, Ethyl Glucuronide and Ketamine and implementation on the Siemens Atellica DT 250 Analyzer. Determine analytical performances of the three ARK homogeneous immunoassays – on the Siemens Atellica DT 250 analyzer compared to performance on the Beckman AU 680.

Methods: Studies were performed at ARK Diagnostics (Fremont, CA) and followed Clinical Laboratory and Standards Institute (CLSI) guidelines. CLSI creates consensus based laboratory standards and guidance recognized by laboratories, accreditors and government agencies to improve testing, speed up product commercialization and streamline regulatory clearance. Validation testing included precision, method comparison (vs. Beckman AU 680 analyzer) with at least 50 positive and 60 negative specimens, analytical recovery, sample carryover, calibration and on-board reagent stability. These immunoassays detect target analytes in human urine.

Results: ARK Fentanyl II: Precision testing at the 1.0 ng/mL cutoff demonstrated overlap between cutoff and controls at 0.5 and 1.5ng/mL. Method comparison showed 100% sensitivity and 100% specificity. Analytical recovery was not determined as the Fentanyl II Assay is a qualitative test. No sample carryover was observed for concentrations up to 3000ng/mL. On board reagent stability was 30 days.

ARK Ethyl Glucuronide II: Total precision ranged from 2.2% to 6.3% CV for concentrations from 125ng/mL to 1750ng/mL. Analytical recovery ranged from between 103.1% to 109.0% for concentrations tested from 200ng/mL to 1400ng/mL. Method comparison showed 100% positive agreement and 100% negative agreement at both the 500 ng/mL and 1000 ng/mL cutoffs. No sample carryover was observed for concentrations up to 100,000ng/mL. On board reagent stability was 30 days.

ARK Ketamine II: Total precision ranged from 3.3% to 5.6% CV for concentrations ≥50 ng/mL to 200ng/mL. Analytical recovery ranged from 104.8% to 112.0% for concentrations tested from 200-400ng/mL. Method comparison showed 100% positive agreement and 100% negative agreement at the 50 ng/mL cutoff and 100% positive agreement and 98.7% negative agreement at the 100 ng/mL cutoff.

No sample carryover was observed with concentrations up to 100,000 ng/mL. On board reagent stability was 30 days.

Discussion: The second-generation ARK urine drug testing assays demonstrated robust analytical performance on the Siemens Atellica DT 250 analyzer, yielding results comparable to the reference platform. These findings support the use of ARK's assays on the Atellica DT 250 analyzer for labs needing high quality, accurate drug screening for fentanyl, ethyl glucuronide or ketamine.

Disclosure

Yes, I, or a member of my immediate family, has a financial interest to disclose.

Conflict of Interest

Authors are salaried employees of ARK Diagnostics

P-224

In Vitro Metabolism Comparison of Ortho-, Meta-, and Para-Methylfentanyl Using Recombinant CYP450 Enzymes

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Abstract

Introduction: Novel psychoactive substances continue to emerge with limited characterization, particularly regarding metabolism. Fentanyl is primarily metabolized by CYP3A4 through oxidative *N*-dealkylation of the piperidine ring to form norfentanyl, although additional phase I biotransformations, such as hydroxylation and *N*-oxide formation, are also reported. Structural differences, including positional isomerism, may influence both pharmacological activity and metabolic pathways. *Ortho*, *meta*, and *para*-methylfentanyl differ only by the position of the methyl group on the aniline phenyl ring, but this positionality may lead to differences in metabolism and enzyme involvement. This study aimed to compare the *in vitro* metabolism of these three isomers and identify the CYP isoforms responsible for their biotransformation.

Objectives: This study aimed to compare the *in vitro* metabolism of *ortho*, *meta*, and *para*-methylfentanyl and to identify the CYP450 isoforms responsible for their biotransformation, with a focus on differences in metabolite formation and enzyme specificity between isomers.

Methods: Methylfentanyl isomers were individually assessed *in vitro* using certified reference materials, recombinant bactosomes, regeneration systems, and phosphate buffer. Initial metabolism experiments were conducted using pooled human liver microsomes (HLM) incubated at 37 °C for two hours using optimized reaction conditions. Reactions were quenched with cold acetonitrile containing fentanyl-D_5 as the internal standard before dilution with mobile phases and analysis using LC-QTOF/MS. Data was acquired in positive electrospray ionization mode using data-dependent acquisition.

Following identification of metabolites, optimized recombinant CYP reactions using CYP3A4, CYP2D6, and CYP2C19 were analyzed after incubating at 0, 30, 60, and 120 minutes. Inhibition studies were conducted in triplicate using CYP-specific inhibitors to confirm metabolite formation from each isoform.

Results: Pooled HLM studies revealed seven proposed metabolites for *ortho*-methylfentanyl and five for both *meta*-and *para*-methylfentanyl. *Ortho*-methylnorfentanyl was confirmed using a reference standard, while the remaining metabolites were presumptively identified based on accurate mass measurements within 5 ppm for the precursor and MS/MS fragmentation products within 10 ppm.

CYP3A4 was the primary enzyme responsible for *N*-dealkylation for all three isomers, producing the corresponding methyl norfentanyl metabolite as the most abundant product. CYP2C19 favored the formation of *ortho*-methyl norfentanyl for the *ortho*- isomer, while hydroxylation of the methylated aniline ring was the dominant pathway for the *meta*- and *para*- isomers. CYP2D6 showed a similar trend, with hydroxylation of the phenethyl group as the most prominent metabolite for *ortho*-methylfentanyl and hydroxylation of the methylated aniline group for the *meta*- and *para*- isomers. Inhibition studies resulted in decreased metabolite formation, supporting the involvement of these enzymes in the observed biotransformations.

Discussion: Differences were observed in metabolite abundance between isomers. CYP3A4 remains the primary enzyme driving metabolism of all three methylfentanyl isomers through *N*-dealkylation, consistent with fentanyl. However, the position of the methyl group influences the predominant metabolite species formed. CYP2D6 and CYP2C19 showed different roles depending on the isomer, particularly in hydroxylation reactions. These findings highlight the impact of small structural differences on metabolism and reinforce the need to evaluate positional isomers individually. These findings strengthen forensic interpretation by demonstrating isomer-dependent metabolic differences, supporting the identification of multiple *o/m/p*-methylfentanyl metabolites in casework where parent compounds may be absent.

Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

P-225

Evaluation of Long-Term Nitazene Stability in Preserved Whole Blood Stored at Different Temperatures

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Abstract

Introduction: Due to increasing prevalence of nitazene opioids, analysts must consider optimal sample storage conditions before analysis of these substances. This is exceedingly important as samples are often shipped to laboratories, may experience delays between receipt and testing, and may require re-analysis months later. Several studies and case reports have suggested that nitazenes, particularly nitro-containing analogs, may exhibit poor stability. As such, further characterization of nitazene stability is needed to inform appropriate storage practices and to determine the impact of stability on the interpretation of results.

Objectives: The purpose of this study was to evaluate the stability of seven nitazene analogs in preserved whole blood stored at four different temperatures (frozen, -20°C; refrigerated, 4°C; ambient, ~20°C; elevated, 35°C).

Methods: The analytes included in the method were metodesnitazene, 4'-OH nitazene, 5-methyl etodesnitazene, *N*-pyrrolidino etonitazene, *N*-piperidinyl etonitazene, isotonitazene, and protonitazene over a working range of 0.5–100 ng/mL (4'-OH nitazene semi-quantitative). Metodesnitazene-D_4 and isotonitazene-$^{13}C_6$ (5 ng/mL) were utilized as internal standards.

Samples were prepared in batches at 1.5 ng/mL or 50 ng/mL of fortified analyte in pooled bovine blood (n=3 sources) preserved with 0.1% sodium fluoride/0.2% potassium oxalate (w/v). Samples were aliquoted into microcentrifuge tubes and stored in four separate conditions: frozen, refrigerated, ambient (uncontrolled), and elevated. Samples were prepared and analyzed using a validated solid-phase extraction (SPE) protocol and liquid chromatography tandem mass spectrometry (LC-MS/MS) method using an Agilent LC-MS/MS and a Poroshell EC-C18 column. Samples from each condition and concentration were extracted in duplicate alongside a freshly prepared calibration curve and quality controls (QC) at designated time points. This evaluation was completed on days 0, 1, 2, 3, 7, 14, 22, and 28, with additional time points in progress for a total period of 6 months. Stability was evaluated by quantitative accuracy and relative peak area ratios relative to time zero (t_0).

Results: Samples exhibited variability across all conditions and time points. Interestingly, increased stability trends were evident for frozen and elevated conditions which are pending further investigation. Metodesnitazene and 5-methyl etodesnitazene remained relatively stable throughout the first month across conditions. However, other nitazenes showed decreased response under refrigerated conditions after several weeks, and a marked decrease in response under ambient conditions after one week. By two weeks, the 1.5 ng/mL samples under ambient conditions resulted in detectable response for protonitazene below the limit of quantitation, however other nitro-containing analytes were not detected.

Discussion: The variability observed across conditions highlights the complexity of nitazene stability. The relative stability observed for metodesnitazene and 5-methyl etodesnitazene compared to others aligns with previous literature suggesting reduced stability for nitro-group-containing nitazenes. Decreased response under refrigerated conditions and rapid loss at ambient temperature indicate that common storage conditions may not adequately preserve all analytes. The apparent stability in frozen and elevated conditions warrants further investigation. Generally, these results indicate that further research on stability of nitazenes continues to be warranted, and caution is necessary when quantitating nitazenes in samples that have been stored for long or unknown periods of time.

Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

P-226

In vitro Phase I Metabolic Profiling of Synthetic Cathinones 3-MMC, *N*-Isopropyl Butylone, *N*-Cyclohexyl Methylone, and Alpha-PiHP

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Abstract

Introduction: The worldwide proliferation of synthetic cathinones poses a threat to human health, and adequate means of detecting these emerging drug threats are essential to the modern forensic toxicologist. Xenobiotic biotransformation is an indispensable source for biomarkers of exposure to these drugs. In this research, *in vitro* metabolism studies were conducted to yield biomarkers of exposure to four novel synthetic stimulants.

Objectives: The goal of this research was to profile the Phase I metabolic pathways of four synthetic cathinones: 3-methylmethcathinone (3-MMC), *N*-isopropyl butylone (*N*-iPB), N-cyclohexyl methylone (N-cHM), and alpha-pyrrolidinoisohexanophenone (alpha-PiHP). Ultimately, this work aimed to uncover Phase I metabolites which could reliably indicate exposure to these drugs in forensic toxicological analysis.

Methods: Phase I metabolites were generated *in vitro* via incubation of drug standards with human liver microsomes (HLMs). The incubation reaction mixtures were analyzed via liquid chromatography with quadrupole-time-of-flight mass spectrometry (LC-QTOF-MS). Phase I metabolites were identified from the acquired data with the aid of SCIEX MetabolitePilot 2.0.4 software. The structures of each metabolite were elucidated from their TOF MS/MS spectra. Sample mining of authentic specimens containing the drugs of interest was performed via LC-QTOF-MS to confirm the presence of the proposed Phase I metabolites *in vivo*.

Results: Six phase I metabolites of 3-MMC were identified in HLM incubation mixtures. Of these, two metabolites corresponding to beta-keto reduction and N-dealkylation of 3-MMC were identified across four authentic oral fluid samples. Seven phase I metabolites of *N*-iPB were identified in HLM incubation mixtures. Of these, three metabolites corresponding to beta-keto reduction, *N*-dealkylation and demethylenation of *N*-iPB were identified across ten samples including whole blood, urine, and vitreous humor. Eight phase I metabolites of *N*-cHM were identified in HLM incubation mixtures. Of these, two metabolites corresponding to beta-keto reduction and aliphatic hydroxylation of *N*-cHM were identified in one sample of whole blood. Nine phase I metabolites of alpha-PiHP were identifiedin HLM incubation mixtures. Of these, five metabolites corresponding to beta-keto reduction, pyrrolidine oxidation, and aliphatic hydroxylation of alpha-PiHP were identified across five authentic whole blood samples.

Discussion: The observation of some predicted metabolites in authentic samples containing parent analytes was consistent with the original hypothesis. Ketone reduction metabolites were consistently detected at the highest peak areas of all metabolites in authentic samples where metabolites were detected. Based on these results, beta-keto reduction products of the synthetic cathinones profiled appear to be the most reliable biomarkers of exposure for these drugs. Additionally, this work profiles the phase I metabolic pathways of *N*-iPB and *N*-cHM for the first time. These are two NPS cathinones identified as emerging threats in the United States by the CFSRE's NPS Discovery program within the past four years. This work represents a preliminary exploration of the metabolism of these drugs and proposes metabolic pathways which may form the basis of future research into the metabolism and pharmacology of these and other emerging synthetic cathinones.

Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

P-227

Legal Responses to New Psychoactive Substances: Impact of the REITOX Network and Different Regulatory Approaches in Europe

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Abstract

Introduction: The unpredictability of new psychoactive substances (NPS), designed to evade existing legislation, challenges drug control and requires rapid and adaptive regulatory responses. Within the European Union (EU), the REITOX network (Réseau Européen d´Information sur les Drogues et les Toxicomanies) and the EU Early Warning System (EWS) support monitoring, risk assessment, and scheduling harmonization. However, countries operating outside this system have heterogeneous regulatory approaches. Understanding disparities in responsiveness, legal certainty, and scientific grounding across Europe is therefore essential.

Objectives: To comparatively analyze the national legal frameworks governing the control of psychoactive substances across European countries both within and outside the REITOX network. By mapping geopolitical trajectories, the study explores how various regulatory systems influence the ability to detect and control emerging substances.

Methods: The analysis focused on REITOX countries (EU Member States, Norway, and Türkiye) within the EWS and on strategically relevant non-REITOX countries, selected based on their significance for the broader European drugs monitoring and regulatory landscape, grouped into four geopolitical trajectories: the Western Balkans, the United Kingdom (UK), Moldova and Ukraine, and Russia and Belarus. Data were collected from primary legal sources, including national drug laws, ministerial regulations, scheduling lists, and reports from the European Union Drugs Agency and the United Nations Office on Drugs and Crime. Legal and institutional frameworks were analyzed, including classification and scheduling models, procedures, and timelines for updating controlled substance lists. The project was partially funded by the Ministry of Health through eLEX-NPS project (CUP I85E25001230005).

Results: Within REITOX countries, EWS-supported systems ensure systematic detection, but responsiveness varies: legislative procedures may delay control by 6–12 months, whereas administrative and emergency measures enable intervention within weeks. In countries like the Netherlands, Hungary, and the Baltic States, temporary control mechanisms serve as regulatory bridges until formal scheduling. While most countries rely on individual substance listing, some adopt generic or analogue-based approaches (e.g., Germany, Ireland), allowing broader and faster coverage of emerging compounds. Outside REITOX, fragmentation is more evident. Some Western Balkans countries have irregular updates, like Bosnia and Herzegovina, which had no revisions from 2011 to 2025 before adding over 100 NPS. Conversely, Serbia demonstrates more dynamic updating, Russia and Belarus use centralized rapid-scheduling models.

Discussion: The findings highlight a heterogeneous but structured European regulatory landscape, characterized by the active role of the REITOX network and by European countries aligning with it to varying degrees. The analysis outlines a range of regulatory paths, from gradual alignment in Western Balkans, Moldova, and Ukraine, to post-integration decoupling in the UK, to more centralized systems in Russia and Belarus. Systems outside REITOX, like the UK, have high regulatory capacity but may be limited in responsiveness due to less participation in European information-sharing. While international or REITOX standards offer an important reference point, their adoption does not necessarily ensure consistent operational effectiveness, especially where updates are irregular or institutions are fragmented. The results suggest that regulatory responsiveness relies more on interactions among structural elements like legal framework, update mechanisms, and institutional coordination than on formal standardization. These findings emphasize the need for improved multilevel coordination and adaptable scheduling to close gaps and safeguard public health across Europe.

Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

P-228

Withdrawn

Withdrawn

P-229

Monitoring the Shift in Québec's New Psychoactive Substances (NPS) Detections: Findings from 2019–2025

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Abstract

Introduction: The market for new psychoactive substances (NPS) is constantly evolving and highly unpredictable, as clandestine laboratories frequently modify the chemical structures of abused substances to evade the legal framework. Since 2019, our laboratory has relied on a dynamic analytical method to monitor these shifting market trends (Garneau et al. (2021) Forensic Science International, 318:110595). New targets are identified through an integrated emerging drug detection system, which combines internal untargeted analytical screening (e.g., GC-MS, QToF) with data from external partners, including Health Canada (drug seizures), public health agencies, and drug checking services. These collaborative efforts allow the timely identification of emerging substances and their integration into the dynamic analytical method.

Objectives: NPS prevalence in casework was evaluated from 2019 to 2025, with a specific focus on trends identified for synthetic opioids and benzodiazepines.

Methods: Blood and urine samples were extracted by protein precipitation, using a mixture of acetonitrile and acetone. The resulting diluted supernatant was analyzed by LC-MS/MS in multiple reaction monitoring (MRM) mode. The procedure was accredited under ISO 17025 and the Standards Council of Canada Forensic Requirements and Guidance. As a dynamic analytical system, the analytes panel was continuously updated: newly emerging NPS were incorporated upon market appearance, while compounds no longer detected over extended periods were removed. The screening panel expanded from an initial 55 NPS and metabolites in 2019 to 68 by December 2025.

Results: In 2019, one or more NPS were detected in 4.5% of antemortem cases and 0.8% of postmortem cases. By 2025, these figures were 2.5% and 3.4% respectively, marking a nearly fivefold increase (2.6%) in postmortem detections.

Between January 2020 and December 2025, synthetic benzodiazepines and opioids emerged as the most prevalent NPS. Amongst benzodiazepines, detections peaked for flubromazolam in early 2020 (n=138), followed by etizolam (n=204) and flualprazolam (n=153) in late 2021. Both then declined, leaving the field open for bromazolam, which reached its highest prevalence in late 2023 (n=135).

Regarding synthetic opioids, fluorofentanyl reached its peak in late 2024 (n=72) following its inclusion in the analytical panel in 2021. Ortho-methylfentanyl was first detected in 2025, immediately following its inclusion in the panel. Medetomidine, identified as an adulterant, has been detected frequently since its addition in late 2025, with 22 cases recorded by year-end. These three substances are often found in combination with fentanyl, which remained

a significant presence throughout the period (n=218) despite a slight decline in detections between 2022 and 2025. Within the nitazene family, isotonitazene (n=36) peaked in late 2020, while protonitazene (n=48) reached its maximum in late 2022 before declining.

Bromazolam, fluorofentanyl, and dexmedetomidine emerged as the primary NPS detected in 2025, alongside flubromazepam, etizolam, and carfentanil.

Discussion: These observations highlight the rapid volatility of the synthetic drugs market, where specific compounds emerge and predominate before being replaced with new variants. A proactive approach is essential to keep pace with this continuous turnover. Furthermore, these data show that drug markets differ in between regions: while most Canadian provinces have been hit with an opioids crisis, synthetic benzodiazepines have always been more popular in the province of Québec.

Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

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Retrospective Time-of-Flight (ToF) Screening of Forensic Toxicology Casework to Identify Novel Psychoactive Substances in Alabama

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Abstract

Introduction: One challenging aspect of forensic toxicology is keeping pace with emerging drug trends and novel psychoactive substances (NPS), which are often unregulated and rapidly appear and disappear from the illegal drug market. Current compounds of interest in Alabama include cychlorphine, an opioid recently associated with several fatalities in surrounding states; mitragynine, a plant-derived compound that is schedule I in Alabama but remains accessible in neighboring states; and compounds found in kava, a plant possessing pharmacological properties similar to alcohol. At the Alabama Department of Forensic Sciences (ADFS), retrospective reprocessing of previously acquired time-of-flight (ToF) data was evaluated to search for emerging compounds of interest.

Objectives: To identify NPS through a retrospective targeted screening of ToF data files originally acquired from June 2025 to March 2026.

Methods: The “Find by Formula” algorithm in MassHunter Qualitative Analysis B.07.00 software was utilized to screen 1,169 ToF files with an NPS-focused MassHunter Personal Compound Database Library (PCDL). Ten compounds and their metabolites (n= 25) were added to a dedicated PCDL. These included six psychoactive components found in the kava plant, AP-237, 2-methyl AP- 237, desalkylgidazepam, medetomidine, SR- 17018, mitragynine, phenazolam, cychlorphine, and ketamine. Each PCDL entry contained compound name, chemical formula, exact mass, and when available, retention time. The “Find by Formula" algorithm scores compounds by accurate mass, retention time, isotopic abundance, and isotopic spacing. Samples were further evaluated for chromatographic peak quality. The positive results detected were organized into the following categories: positive, positive with review, and presumptive positive. Presumptive results indicate the lack of available retention times for comprehensive assessment.

Results: 173 putative identifications were observed across 1,169 data files from 109 antemortem and postmortem cases. The compounds identified most frequently were: mitragynine (16%), mitragynine pseudoindoxyl (14%), kavain (12%), yangonin (12%), and ketamine (11%). AP-237, 2-methyl-AP-237, desalkyldiazepam, 3-hydroxy-desalkylgidazepam, SR-17018, 5,6-dichloro-nor-orphine, alpha-hydroxy-phenazolam, chlorphine, and cychlorphine, were not detected.

Discussion: Approximately 39% of the positive results were associated with mitragynine and its related metabolites, while 32% were attributed to the psychoactive components found in the kava plant. Notably, cychlorphine was not detected however; nor-orphine, a reported metabolite, was detected in three cases and warrants further evaluation. Due to a lack of retention times, presumptive positives required more rigorous evaluation with tighter criteria for mass accuracy and peak shape. These findings illustrate the value of retrospective ToF data analysis as an adaptable strategy for uncovering emerging NPS trends, enabling laboratories to proactively refine analytical scopes within a dynamic drug landscape.

Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

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Mistaken Identity: A Prevalence Study of Bromazolam in the American Great Lakes Region

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Abstract

Introduction: Bromazolam (BRZ) is a novel psychoactive substance benzodiazepine drug that has recently emerged within the illicit drug supply in the United States. BRZ is not approved by the U.S. Food and Drug Administration and was added to Schedule I by the Drug Enforcement Administration in March of 2026. Seized drug reports have indicated its distribution in drug mixtures or as counterfeit 'Xanax.'

Objectives: Our objective was to identify the drug trends associated with BRZ in the Great Lakes region from our oral fluid toxicology data. Specifically, we examined what illicit drugs were commonly associated in BRZ-positive cases, how frequently other prescription benzodiazepines were present in these cases, and where these cases took place in this region of the United States.

Methods: Oral fluid specimens were collected and submitted to Forensic Fluids Laboratories by our clients. In general, samples were submitted from child protection agencies, probation departments, treatment and specialty courts, and healthcare partners. BRZ-positive sample results were examined between August 2024 and November 2025. Samples were first screened using ELISA (Benzodiazepine Direct ELISA kit, Immunalysis, target analyte for the kit: oxazepam, 5 ng/mL cutoff), and presumptive positive samples were confirmed using LC-MS-MS methods (BRZ cutoff 1 ng/mL). Illicit drug data (methamphetamine, cocaine, 6-monoacetylmorphine, fentanyl) and other benzodiazepine content from samples, medication lists, and associated geospatial data were gathered to indicate prevalence, identify use trends, and map BRZ use.

Results: BRZ was found in 219 of 4,579 samples that screened positive for benzodiazepines (4.8%). Approximately half (109, 49.8%) of cases contained at least one other illicit substance. The most common substance identified in BRZ-positive samples was cocaine (73, 33.3%), followed by fentanyl (56, 25.6%), methamphetamine (38, 17.4%), and 6-monoacetylmorphine (11, 5.0%). A subset of samples (45, 20.5%) listed at least one prescription benzodiazepine in the test requisition paperwork. Alprazolam was the most listed prescription benzodiazepine (25, 55.6% of BRZ-cases with benzodiazepine prescriptions), followed by clonazepam (17, 37.8%). Many of these samples reported negative for the listed medication (17, 37.8%). Mapped data of BRZ-cases indicated widespread use in the Great Lakes region, largely identified in Michigan and Ohio with more limited cases in Wisconsin and Indiana. The overall incidence of BRZ-positive cases declined over this time in this region.

Discussion: BRZ was found with illicit drugs and by itself in about an equal share in submitted oral fluid samples. While fentanyl is frequently reported in literature to be found with BRZ, our data indicated cocaine to be the most prevalent co-occurring substance. The other half of samples without illicit substances identified presents a unique challenge in clinical settings which frequently rely on instant screening devices for benzodiazepine compliance. Our data indicated that nearly two of every five BRZ-cases with listed prescription benzodiazepines did not contain the listed prescription. Cross-reactivity of BRZ in immunoassay-based tests may confound interpretations if used alone, leading to incorrect assessments of appropriate benzodiazepine use. The declining incidence of BRZ (notably, in advance of its Schedule I assignment) may indicate the beginnings of a shift in drug use trends for this region. Oral fluid was an effective tool for detecting this substance.

Disclosure

Yes, I, or a member of my immediate family, has a financial interest to disclose.

Conflict of Interest

Authors are paid salary by Forensic Fluids Laboratories

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Prevalence and Toxicological Significance of 3-CMC and 4-CMC: A Retrospective Study of Over 250 Forensic Cases (2011–2026)

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Abstract

Introduction: Synthetic cathinones remain a highly dynamic class of New Psychoactive Substances (NPS) across the global drug market. In Europe, chlorinated derivatives such as 3-chloromethcathinone (3-CMC) and 4-chloromethcathinone (4-CMC) have gained substantial popularity, frequently appearing in drug seizures and forensic cases. Their widespread availability has led to a notable increase in health-related emergencies and forensic investigations, particularly regarding drug-impaired driving (DUID), posing a significant threat to public road safety.

Objectives: The aim of this study was to provide a comprehensive analysis of the occurrence and concentrations of 3-CMC and 4-CMC in biological specimens collected over a 15-year period in Poland, evaluating their prevalence in various forensic contexts.

Methods: Toxicological screenings allowed for the simultaneous detection of chloromethcathinones alongside a broad spectrum of classical drugs and other NPS. The quantitative analyses of 3- and 4-CMC were performed primarily on blood (with occasional urine samples) using liquid chromatography-tandem mass spectrometry (LC-MS/MS). The analytes were isolated from 0.2 mL of biological material by liquid-liquid extraction (LLE) with ethyl acetate under alkaline conditions (pH 12).

Results: The study identified 80 cases of 3-CMC and 199 cases of 4-CMC, with 15 cases involving both substances. While polydrug use predominated, 3-CMC was the sole substance in 26 cases, and 4-CMC in 56 cases. The substances were most frequently identified in cases related to DUID, traffic accidents, drug possession, violent crimes, and fatalities. Co-detected substances included THC, amphetamine, benzodiazepines, cocaine, MDMA, other cathinones, synthetic cannabinoids, and the synthetic opioid. Blood concentrations for 3-CMC ranged from traces (<1 ng/mL) to 1287 ng/mL (mean: 34 ng/mL; median: 8 ng/mL), while 4-CMC ranged from traces (<1 ng/mL) to 2084 ng/mL (mean: 51 ng/mL; median: 14 ng/mL). The highest concentrations for both compounds were recorded in fatal intoxication cases. To better evaluate the impact on psychomotor performance, a sub-group of mono-chloromethcathinone DUID cases (where no other psychoactive substances were detected) was analyzed (3-CMC cases; n=18, mean: 15 ng/mL, median: 9 ng/mL) and (4-CMC cases; n=26, mean: 42 ng/mL, median: 25 ng/mL). In five traffic accidents where only 4-CMC was detected, the mean and median were 165 and 107 ng/mL, respectively. Among 38 DUID cases in which no other substances (including alcohol) were detected, dilated pupils were observed in only 6 instances, and a slow pupillary light reflex in four. No other characteristic symptoms were observed. Despite this, in 22 cases, the physician collecting the blood sample suspected the use of psychoactive substances.

Discussion: The high prevalence of 3-CMC and 4-CMC underscores their significant role in the European drug landscape. The consistently higher concentrations observed for 4-CMC compared to 3-CMC across all case categories suggest potential differences in typical dosing or pharmacokinetics. Despite observable symptoms of impairment in many subjects, establishing definitive concentration-effect correlations remains complicated. This difficulty stems from the high frequency of polydrug consumption and the instability of chloromethcathinones in blood, which must be carefully considered during the interpretation of toxicological results.

Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

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Positional and Enantioselective Discrimination of Methylmethcathinones (MMC): LC–MS/MS Analysis of 2-, 3- and 4-MMC in Whole Blood

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Abstract

Introduction: The synthetic cathinones 2-MMC (2-methylmethcathinone), 3-MMC (3-methylmethcathinone) and 4-MMC (4-methylmethcathinone) are new psychoactive substances (NPS) increasingly encountered in forensic and clinical toxicology.

These compounds exhibit two types of isomerism: positional (constitutional) and optical. Positional isomerism results from the presence of a methyl substituent at the 2-, 3-, or 4-position of the aromatic ring, whereas optical isomerism arises from the existence of mirror-image enantiomers, giving rise to two stereoisomeric forms. Differentiating positional and enantiomeric forms is essential because all three cathinones exhibited stereoselective properties and the relocation of the methyl group from the para to the ortho position shifts the pharmacological profile. For example, while (R)-4-MMC drives dopamine transporter mediated stimulation, the (S)-enantiomer mediates entactogenic effects via the serotonin transporter.

Conventional analytical methods commonly used in toxicology (GC-MS and achiral LC-MS/MS) cannot discriminate between these isomers, limiting interpretation.

Objectives: The present study aimed to develop and validate a chiral LC-MS/MS method for the simultaneous positional 2-, 3- and 4-MMC and enantioselective discrimination of 3- and 4-MMC in whole blood.

Methods: Whole blood samples underwent protein precipitation with Acetonitrile (at -20°C), phospholipid removal (Phree™ columns), evaporation, and reconstitution in methanol prior to analysis in a LC-MS/MS (Waters Acquity UPLC). Chromatographic separation was achieved using a Lux® 3 µm AMP chiral column (Phenomenex) under optimized conditions. Mobile Phase A contained LC–MS grade water and 0.025% (v/v) aqueous ammonia, while Mobile Phase B contained LC–MS grade methanol with 0.025% (v/v) aqueous ammonia.

Method validation was performed according to ANSI/ASB Standard 036, including assessment of selectivity, carryover, matrix effects, linearity, limits of detection (LOD) and quantification (LOQ), precision, and accuracy.

Results: Positional discrimination of all MMC isomers was achieved, along with baseline separation of 3-MMC and 4-MMC enantiomers. Racemic reference standards enabled identification of the (R)- and (S)-enantiomers of 3-MMC and 4-MMC. For 2-MMC, only a single peak was observed for the available reference material, preventing the assessment of its enantiomeric composition and the assignment of absolute configuration. The method showed good sensitivity, selectivity and linearity within forensic concentration (non-fatal and fatal) previously published (5-1000 ng/mL), with no significant matrix effects observed. The method was successfully applied to authentic forensic cases, in which 2-MMC was detected in whole blood samples. No 3-MMC or 4-MMC cases were detected.

Discussion: The developed chiral LC-MS/MS method was fully validated according to ANSI/ASB Standard 036 and provides a robust tool for the positional and enantioselective discrimination of MMC isomers in whole blood. The method was applied to routine post-mortem samples, being already detected four positive cases of 2-MMC.

Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

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Integrated Multi-Analytical Approaches for the Detection of New Psychoactive Substances: Identification of a New Brominated Synthetic Cannabinoid in Herbal Seizure Samples

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Abstract

Introduction: New psychoactive substances (NPS) represent a growing challenge for forensic and toxicological laboratories due to their continuous structural modification, which aims to evade legal control. Among them, synthetic cannabinoids (SCs) are the most diverse and frequently detected class, often exhibiting high potency compared to Δ^9-THC. In recent years, brominated analogs have emerged as an important structural strategy to circumvent legislation. In this context, this study highlights the importance of a multi-analytical approach for the reliable identification and structural elucidation of emerging NPS. Accordingly, this study reports the identification and structural elucidation of a novel synthetic cannabinoid, MDMB-5′Br-PINACA, which was detected in three seized herbal materials for the first time worldwide.

Objectives: The aim was to identify and structurally characterize MDMB-5′Br-PINACA in seized herbal samples and to evaluate the chemical profile of the materials using an integrated multianalytical approach.

Methods: Following the preliminary screening of seized materials, three seized herbal materials were identified as potentially containing a newly emerging NPS and were therefore selected for subsequent investigation. Samples were extracted with organic solvents and initially screened using gas chromatography coupled to a triple-quadrupole mass spectrometer (GC-MS/MS) and liquid chromatography coupled to a quadrupole time-of-flight mass spectrometer (LC-HRMS). The target compound was isolated by semipreparative LC. Structural elucidation was further achieved using nuclear magnetic resonance (NMR) spectroscopy, including 1D and 2D experiments, in accordance with a multimodal analytical workflow.

Results: The seized materials contained complex mixtures of NPS, including SCs (such as 5F-ADB and MDMB-4en-PINACA), designer benzodiazepines, and a nitazene. During the initial screening of these materials, a compound was consistently detected whose mass spectrum showed partial similarity to known brominated SCs, while exhibiting a molecular ion at m/z 437, suggesting the presence of a structurally related but distinct substance. This finding prompted further investigation, which revealed that a previously unknown compound was present in all samples and was subsequently identified as MDMB-5′Br-PINACA. GC-MS/MS and LC-HRMS

data indicated the presence of a brominated synthetic cannabinoid; in particular, this conclusion was supported by characteristic isotopic patterns and fragmentation profiles. Moreover, HRMS confirmed the molecular formula and, additionally, suggested structural similarity to known brominated SC analogues. Subsequently, NMR analysis confirmed that MDMB-5′Br-PINACA is a methyl ester analog of ADB-5′Br-PINACA, in which the amide group is replaced by an ester functionality. Furthermore, extraction experiments using different solvents were conducted to evaluate potential artifacts. These experiments ruled out artifact formation during sample preparation, thereby confirming that the compound was genuinely present in the seized materials. In addition, variations in abundance among samples indicated a heterogeneous distribution of the substance.

Discussion: The findings highlight the increasing complexity and rapid evolution of the chemical complexity of NPS markets, particularly SCs. Bromination appears to be a strategic modification that may preserve or enhance pharmacological activity while complicating detection and regulatory control. The study demonstrates the importance of integrated, multi-analytical approaches for accurately identifying and characterizing emerging substances. The discovery of MDMB-5′Br-PINACA expands current knowledge of emerging SCs and underscores the need for continuous monitoring to support forensic investigations, public health surveillance, and evidence-based drug policy and harm reduction strategies.

Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

P-236

Di-Protonation-Driven Structural Elucidation of 5-Methyl Etodesnitazene Metabolites by LC-ESI± HRMS/MS

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Abstract

Introduction: The rapid emergence of nitazene as highly potent novel synthetic opioids represents a significant growing challenge for public health. Their high potency and extensive structural diversification make timely analytical detection and reliable metabolite identification essential for toxicological screening, biomarker discovery, and case interpretation. Analytical identification of nitazene opioids remains particularly challenging due to their poor fragmentation behavior in conventional LC-ESI± HRMS/MS workflows. Usually, benzylbenzimidazole containing an *N*,*N*-diethylamino moiety typically undergo preferential protonation at the tertiary aliphatic amine, producing mainly low-mass and poorly specific fragments such as diethylamine and *N*,*N*-diethylethanamine. This limits confident structural elucidation of metabolites, especially when differentiating positional biotransformations. Notably, some nitazenes analogues, including 5-methyl etodesnitazene, generate abundant di-protonated ions that may substantially improve fragmentation quality and metabolite assignment.

Objectives: This study aimed to evaluate the analytical relevance of mono- and di-protonated precursor ions for the structural elucidation of 5-methyl etodesnitazene and its metabolites, and to assess the role of di-protonation as a strategy to improve metabolite identification in forensic toxicology.

Methods: The metabolic profile of 5-methyl etodesnitazene was investigated using human hepatocyte incubations and postmortem specimens. Human hepatocytes were incubated with the parent compound to generate phase I and II metabolites, while samples from a confirmed fatal intoxication case were included to verify the relevance of the identified metabolites *in vivo*. Chromatographic separation was performed using a biphenyl-bonded analytical column coupled to LC-ESI± HRMS/MS. To specifically investigate protonation behavior, samples were analyzed in positive-ionization mode using two separate inclusion lists designed to target the theoretical mono-protonated and di-protonated precursor ions of the parent drug and its putative metabolites. Data analysis combined software-assisted targeted and untargeted mining with Compound Discoverer and in silico prediction using GLORYx.

Results: 5-Methyl etodesnitazene produced an intense and highly stable di-protonated molecular ion, often showing greater analytical relevance than the mono-protonated. The mono-protonated precursor mainly generated dominant low-mass fragments at *m/z* 72 and 100, corresponding to diethylamine and *N*,*N*-diethylethanamine, providing limited structural information and increasing the risk of analytical interference. In contrast, fragmentation of the di-protonated ion retained charge on the benzimidazole scaffold, generating highly informative product ions such as *m/z* 267, 239, 145, and 135, which enabled direct localization of metabolic transformations. This was particularly important for distinguishing *N*-deethylation from *O*-deethylation and for confirming

ω-carboxylation at the 5-methyl benzimidazole core. Twenty-five metabolites were identified in hepatocyte incubations, and twenty-one were confirmed in authentic biospecimens. Major pathways included *N*-deethylation, *O*-deethylation, ω-carboxylation, hydroxylation, oxidative deamination, and carbonyl formation. Di-protonated precursor ions were essential for confident assignment of the major biomarkers, particularly *N*-deethyl-5-methyl etodesnitazene, *N*-deethyl-5-carboxy etodesnitazene, and 5-carboxy etodesnitazene.

Discussion: Di-protonation significantly enhances structural elucidation of metabolites by overcoming the poor fragmentation typically associated with mono-protonated tertiary amine-containing opioids. Rather than relying only on low-mass side-chain fragments, di-protonated ions preserve diagnostically useful fragments from the benzimidazole core and aromatic moieties, substantially improving metabolite identification. For 5-methyl etodesnitazene, di-protonation proved to be a decisive analytical advantage for both in vitro and authentic samples. This approach improves biomarker selection and should be systematically considered during HRMS method development for nitazenes and other synthetic opioids showing similar protonation behavior.

Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

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Bridging Biosensing and Gold-Standard Analytics: Preliminary Analytical Evaluation of a MIP-QCM Platform for Fentanyl and Norfentanyl Detection in Human Urine

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Abstract

Introduction: Fentanyl and its primary metabolite, nor-fentanyl, are highly potent synthetic opioids whose monitoring is essential in clinical and forensic toxicology. The increasing prevalence of opioid-related morbidity and mortality has created a demand for rapid and reliable methods capable of detecting these compounds in biological matrices such as urine. Although liquid chromatography-tandem mass spectrometry (LC-MS/MS) remains the analytical gold standard owing to its sensitivity and selectivity, alternative approaches offering rapid, cost-effective, and label-free analysis are increasingly attractive. Quartz crystal microbalance (QCM)-based biosensors enable real-time analyte monitoring, whereas molecularly imprinted polymers (MIPs) improve analytical specificity.

Objectives: This study aimed to develop and optimize a MIP-based QCM biosensor for the selective detection of fentanyl and nor-fentanyl in human urine. Analytical performance was evaluated against a validated quantitative LC-MS/MS method, and applicability was assessed using authentic urine samples.

Methods: MIP layers selective for fentanyl and nor-fentanyl were synthesized directly on QCM crystal surfaces using functional monomers, cross-linkers, and template molecules. Template removal generated binding cavities complementary to the target analytes. Surface characterization by FTIR, SEM, and contact angle measurements confirmed successful MIP formation and template removal. Artificial urine fortified with fentanyl and nor-fentanyl was used during optimization and preliminary validation studies. Samples were diluted with running buffer and analyzed under continuous-flow conditions. Sensor performance was evaluated using triplicate measurements at each concentration level and three independently prepared sensor surfaces. Experimental parameters, including pH, ionic strength, and flow rate, were optimized. The platform exhibited a linear response across 0.5-500 ng/mL for both analytes. Limits of detection (LOD) were estimated using a signal-to-noise ratio of 3:1. Selectivity was assessed against structurally related opioids (morphine, codeine, tramadol, and methadone), selected over-the-counter medications, and common urinary matrix components. The optimized platform was applied to authentic human urine samples (n = 15). LC-MS/MS served as the reference analytical technique, and agreement between methods was evaluated by linear regression analysis.

Results: The MIP-QCM biosensor demonstrated high sensitivity, selectivity, and reproducibility for both analytes. Compared with non-imprinted polymer controls, the MIP-based sensor exhibited enhanced binding affinity and target recognition. Consistent linear responses and good repeatability were obtained across independently prepared sensor surfaces, with coefficients of variation below 15%. No significant interference was observed. The platform successfully discriminated fentanyl and nor-fentanyl from structurally related compounds while maintaining analytical performance in urine matrices. Analysis of authentic urine samples demonstrated strong agreement with the validated LC-MS/MS method, with correlation coefficients of $R^2 = 0.982$ for fentanyl and $R^2 = 0.987$ for nor-fentanyl. No false-positive responses were observed within the evaluated sample set and experimental conditions. Preliminary LOD values were below 2 ng/mL for both analytes. Additional validation studies, including expanded evaluation using authentic urine samples, are ongoing.

Discussion: The proposed MIP-QCM biosensor represents a rapid, label-free platform for fentanyl and nor-fentanyl detection in urine. The strong correlations observed with the quantitative LC-MS/MS reference method support its analytical potential, while successful application to authentic samples highlights its utility as a screening tool in forensic and clinical toxicology. Ongoing studies will further establish analytical performance and support development of biosensing technologies for opioid monitoring.

Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

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Development of a Molecularly Imprinted Polymer (MIP)-Based Quartz Crystal Microbalance (QCM) Biosensor for Detection of Alcohol Biomarkers (EtG/EtS) in Urine

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Abstract

Introduction: Alcohol consumption poses significant risks to public health, traffic safety, and occupational environments, requiring reliable monitoring in clinical and forensic toxicology. While direct measurement of ethanol provides information limited to a short time window due to its rapid metabolism, minor metabolites such as ethylglucuronide (EtG) and ethyl sulfate (EtS) stand out as powerful biomarkers because they can be detected over a longer period. Today, the determination of these compounds is typically performed using highly sensitive methods such as LC-MS/MS and GC-MS; however, these techniques have limitations such as high cost, long analysis times, and reliance on laboratory facilities.

Objectives: The primary objective of this study is to develop and optimize a molecularly imprinted polymer (MIP)-based quartz crystal microbalance (QCM) biosensor platform capable of rapidly, selectively, and sensitively detecting EtG and EtS in urine samples at low cost.

Methods: Molecularly imprinted polymers (MIPs) are synthetic polymers that form structures capable of binding to target molecules with high specificity and are widely used in biosensor applications. In this study, EtG and EtS imprinted MIP films were prepared and immobilized on a QCM chip surface. Surface characterization was performed using Fourier-transform infrared spectroscopy (FTIR), scanning electron microscopy (SEM), atomic force microscopy (AFM), zeta potential measurements, ellipsometry, and contact angle measurements. The selectivity, sensitivity, stability, and repeatability performance of the printed and unprinted sensors were evaluated.

Results: The developed MIP-QCM biosensors demonstrated high sensitivity at the pg/mL-ng/mL range and exhibited reliable analytical performance with a wide linear working range. The system provided an effective response at low concentrations and showed high selectivity against matrix effects. Furthermore, it has been demonstrated that the system offers a viable alternative for on-site analysis applications due to its short analysis time, low cost and portability.

Discussion: Molecularly Imprinted Polymer (MIP) based QCM biosensors represent an innovative approach offering high selectivity for the detection of alcohol biomarkers such as EtG and EtS. The developed system has been shown to serve as an alternative to existing conventional methods due to its fast, sensitive, and low-cost analytical capabilities. Its portable and label-free platform design provides significant advantages for on-site analysis applications. As part of future work, we plan to comparatively validate the performance of the developed sensor in real urine samples using LC-MS/MS validated methods. This validation will more robustly demonstrate the system's analytical reliability and practical applicability.

Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

P-239

Evaluation of a Novel Oral Fluid Collection Device: Drug Recovery, Matrix Effects, and Analyte Stability

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Abstract

Introduction: Oral fluid (OF) testing is increasingly adopted in forensic, clinical, and workplace toxicology due to its non-invasive, directly observed collection and its ability to reflect recent drug use. However, variability in collection device performance remains a key limitation to broader implementation. Pre-analytical factors including drug recovery, matrix effects, and analyte stability are critical to ensuring reliable downstream analysis. A novel OF collection device (Xpeedy®) was developed to enable a single-sample, dual-workflow approach supporting both on-site screening and laboratory-based confirmatory testing.

Objectives: This study evaluated the performance of the Xpeedy® Oral Fluid Collection Device across key parameters, including drug recovery, matrix effects, and analyte stability, in comparison with a FDA 510(k) cleared OF collection device (predicate device).

Methods: Drug-free human OF samples were fortified with target analytes at specific concentrations and collected using the Xpeedy® Oral Fluid Collection Device and the predicate device. Samples were extracted using magnetic solid-phase extraction (mSPE) and analyzed by LC–MS/MS (Shimadzu LCMS-8040).

Drug release was evaluated across 23 analytes (including 9 SAMHSA panel drugs), with recovery assessed at 1 minute, 10 minutes, and 4 hours post-collection (n=4).

Stability was evaluated across the same 23 analytes under room temperature (RT) and 4 °C storage on Days 7, 10, and 14 (n=3), with results expressed as percent bias relative to freshly prepared controls.

Matrix effects were assessed across 48 analytes using a post-extraction spike approach (Matuszewski method), with results reported as percent bias relative to neat standards.

Results: For drug release, Xpeedy® demonstrated rapid recovery across all 23 analytes, achieving >85% recovery within 1 minute of collection. In contrast, the predicate device showed substantially lower recovery even at extended timepoints.

For stability, all 23 analytes collected with Xpeedy® remained within ±20% bias through Day 14 at 4 °C. At RT, 22 of 23 analytes remained stable through Day 14, while THC remained stable through Day 10, exceeding typical SAMHSA expectations for OF stability (±20% bias for ≥5 days). Performance was comparable to that of the predicate device.

For matrix effects, all 48 analytes collected with Xpeedy® were within ±30% bias, indicating minimal ion suppression/enhancement across multiple drug classes. The predicate device demonstrated greater variability, with several analytes exceeding ±30% bias.

Discussion: The Xpeedy® Oral Fluid Collection Device demonstrated robust performance across key pre-analytical parameters, including rapid drug release, minimal matrix effects, and extended analyte stability, which enables the device to be compatible with both point-of-care screening and confirmatory LC–MS/MS workflows, and supports a streamlined, single-collection approach. These features may improve analytical reliability and facilitate broader adoption of OF testing in toxicology applications.

Disclosure

Yes, I, or a member of my immediate family, has a financial interest to disclose.

Conflict of Interest

All authors are employed by CURA Diagnostics Inc. and receive salary support; Jia He holds stock ownership in the company.

P-240

Withdrawn

P-241

Now You See It, Now You Don't: The Rise, Fall, and Return of Para-Fluorofentanyl

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Abstract

Introduction: Para-fluorofentanyl (pFF) is a fentanyl analog synthesized in the 1960s for research purposes by Janssen Pharmaceuticals, categorized as a Schedule I controlled substance in 1986. pFF is a mu-opioid receptor agonist that has a relative potency less than or equal to that of fentanyl and 50 to 100 times that of morphine. pFF was developed, abandoned, rediscovered in the illicit drug market for a brief period in the 1980s, and returned to the illicit drug market in 2016 across the United States. From July 25, 2018, through September 24, 2020, across 36 counties, Michigan (34) and Indiana (2), pFF was not identified in blood from postmortem cases analyzed by the toxicology laboratory at Western Michigan University Homer Stryker M.D. School of Medicine (n = 4,390 cases). From September 25, 2020, to March 30, 2026, an analysis of 15,444 postmortem blood samples confirmed the presence of pFF in 870 cases. Fentanyl was co-detected in 862 of the 870 cases (99%).

Objectives: The objective of this study was to conduct a longitudinal assessment of pFF in our catchment area (Southwest Michigan) during periods of quiescence, reemergence, and now an apparent steady rate.

Methods: A solid-phase extraction and a qualitative liquid chromatography-tandem mass spectrometry (LC-MS/MS) analysis for pFF plus 50 additional opioids and 24 non-opioids were performed on 19,834 postmortem cases. In brief, deuterated internal standards were added to case samples and quality control material, then extracted, dried, reconstituted, and analyzed via LC-MS/MS. Chromatographic separation was accomplished using a biphenyl column maintained at 40°C and electrospray ionization operated in positive-ion mode. Two ion transitions were monitored per targeted analyte. Chromatographic peak retention time, transition mass information, and peak area ratios were used to identify and qualitatively report results.

Results: For a two-year, two-month period, pFF was not identified in any analyzed cases (n=4,390 cases); however, during the subsequent five-year, six-month period, pFF was identified in 870 of 15,444 cases analyzed, demonstrating reemergence after a period of absence. In total, 19,834 postmortem blood specimens were analyzed. Fentanyl was co-detected in 862 of the 870 (99%), and at least one additional targeted drug was identified in all cases. Quantifications performed on 146 postmortem blood specimens sent to an external reference laboratory resulted in para-fluorofentanyl concentrations ranging from 0.09 to 120 ng/mL, with a median concentration of 1.45 ng/mL and a mean of 7.12 ng/mL. Although most concentrations were low, several markedly elevated values were observed.

Discussion: pFF was identified in our postmortem casework after a period of being undetected, followed by a rise, a decline, a reappearance, and now a regular encounter in the Southwest Michigan illicit drug market. pFF is frequently co-detected with fentanyl with concentrations as high as 120 ng/mL, perhaps suggesting in some cases the combination may be purposeful. Toxic and fatal pFF ranges are undefined; therefore, continued surveillance and additional case data are needed to guide forensic interpretation.

Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

P-242

Assessment of Postmortem Urine Fentanyl Detection by Autopsy Dipstick Testing in Accidental Overdose Deaths

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Abstract

Introduction: Accidental overdose deaths have increased markedly over the past two decades, with synthetic opioids, particularly fentanyl, remaining as the lead contributors to mortality. This increasing caseload has placed substantial operational and financial strain on medical examiner offices and forensic toxicology laboratories. As a result, rapid point-of-care urine dipstick testing is frequently used during autopsy as a preliminary triage tool to guide toxicological investigation. However, fentanyl immunoassay-based dipstick tests may demonstrate limited analytical sensitivity and potential cross-reactivity, raising concerns regarding their reliability in postmortem case assessment. This study evaluates whether urine dipstick testing is sufficient for accurately identifying fentanyl-related overdose deaths and appropriately triaging cases for confirmatory toxicological analysis.

Objectives: The objective is to evaluate the concentrations of fentanyl and norfentanyl in postmortem blood and urine specimens in all accidental overdose deaths in San Francisco from 2020 to 2023; compare the concentrations of postmortem urine fentanyl and norfentanyl with the cut-off levels of commonly available commercially marketed urine dipstick tests; assess the diagnostic utility of dipstick testing to correctly identify fentanyl cases, by evaluating whether such rapid tests could meaningfully detect and triage fentanyl-related cases for further comprehensive toxicology testing.

Methods: This retrospective review includes evaluating 1,550 fentanyl-related accidental overdose cases in San Francisco, between January 2020 through December 2023, where both postmortem peripheral femoral blood and urine specimens were available for toxicological analysis using LC-MS/MS. Cases were then categorized into the following 3 categories: blood and urine positive; blood positive, urine none detected; and blood none detected, urine positive. Estimated urine concentrations derived from this data were then compared with the cut-off thresholds used in commercially available urine dipstick tests (e.g., 1, 5, 10, 20, 50, and 100 ng/mL) to determine how many fentanyl-related cases would be missed at each threshold if dipstick testing was used as a primary decisive tool.

Results: Eighty-two of the 1,550 cases detected fentanyl and/or norfentanyl in blood but were undetected in the corresponding urine samples. When comparing estimated urine fentanyl/norfentanyl concentrations against common dipstick cut-off levels at 1 ng/mL and 5 ng/mL cut-offs, 7% and 19% of cases would be false negatives, respectively. At higher thresholds such as 10 ng/mL and 20 ng/mL, 24% and 25% of cases would be false negatives, respectively. At very high thresholds (50 ng/mL and 100 ng/mL), false negatives increased dramatically to 51% and 61%, respectively.

Discussion: The study demonstrates that postmortem urine dipstick tests for fentanyl are not sufficiently reliable to serve as stand-alone indicators of fentanyl involvement in accidental overdose deaths. False negatives-- where fentanyl was present in blood yet below the dipstick detection threshold in urine-- raise concerns about using such rapid tests for triage decisions regarding further toxicology testing or determinations of cause and manner of death. These findings caution forensic professionals against over-reliance on urine dipsticks in isolation and support the continued use of comprehensive testing methods, like LC-MS/MS, for accurate detection and quantification.

Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

P-243

Death with Dipyanone: A Postmortem Polydrug Case in Montana

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Abstract

Introduction: Dipyanone is an unscheduled novel synthetic opioid (NSO) that is similar to methadone in structure, μ-opioid receptor potency, and abuse potential. Dipyanone has a lower potency when compared to most other NSOs. Like other opioids, dipyanone may cause central nervous system (CNS) depression, including respiratory depression and hypotension. These symptoms could become fatal, especially in combination with other CNS depressants. The current literature on dipyanone is limited.

Case History: A 27-year-old male was found unresponsive with agonal breathing. Lifesaving measures were unsuccessful, including Narcan administration. Multiple baggies containing suspected fentanyl were found in the decedent's pocket and injection sites on both arms were noted. The decedent was known to use many drugs and "had recently stated that he was trying to use other drugs to get away from drugs."

Objectives: To present the findings of a postmortem case containing dipyanone in a polydrug death and emphasize the role of multidisciplinary forensic analysis in identifying novel psychoactive substances (NPS).

Methods: Postmortem specimens were collected at autopsy at the Montana Forensic Science Division (FSD) in Missoula, MT. Specimens included femoral and heart blood, vitreous, and urine. Femoral blood was screened for volatiles using HS-GC-FID, and for drugs qualitatively by ELISA and LC-QToF-MS. The urine sample was qualitatively screened by ELISA and LC-MS/MS. Femoral blood was sent to National Medical Services Labs (NMS) and cardiac blood to the Center for Forensic Science Research and Education (CFSRE) for NPS quantitations.

Results: Femoral blood toxicology was negative for volatiles and positive for: 7-aminoclonazepam, bromazolam (60 ng/mL, NMS), desalkylflurazepam (52 ng/mL, NMS), dipyanone, and fluoromethamphetamine (isomer not determined). Urine toxicology revealed 7-aminoclonazepam, amphetamine, benzoylecgonine, bromazolam, desalkylflurazepam, methamphetamine, oxazepam, and delta-9 THC-COOH. The dipyanone quantitation from CFSRE is currently pending.

Chemical analyses of the baggies confirmed dipyanone, bromazolam, methiodone, and 2-fluoromethamphetamine.

Discussion: This report documents the first known case containing dipyanone in Montana. Dipyanone's similarity to methadone suggests it may be used experimentally as an alternative to other opioids and to curtail withdrawal. Subjective user blog comments indicate they use dipyanone "as a method of quitting opioids" and describe it as "amazing for maintenance".

Dipyanone was found with other NPS and designer benzodiazepines. This extensive combination demonstrates the complexity of modern polydrug use and challenges they present

for detection and interpretation. Based on the postmortem redistribution (PMRD) characteristics of methadone, dipyanone may also be susceptible to PMRD. With the combined effects of these drugs, the cause of death cannot be attributed to any one specific substance. The manner and cause of death for this individual were accidental mixed drug toxicity.

While routine toxicology testing identified many of the NPS, assistance from multiple forensic disciplines aided in a more comprehensive investigation. Preliminary toxicology results prompted the state's coroner liaison to request submission of drug evidence, allowing Drug Chemistry to analyze the baggies. The Medical Examiner and Toxicology Section coordinated additional drug testing via reference lab and CFSRE.

This case demonstrates the limitations of routine toxicology screening in detecting NPS. It also emphasizes successful coordinated communication among forensic disciplines, which is key to providing thorough results for both public health and family closure.

Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

P-244

Metformin-related Deaths Are Naturally Complicated

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Abstract

Introduction: Metformin (Glucophage) is an oral medication prescribed to manage type II diabetes (DM) by lowering blood sugar levels but has a significant risk: lactic acidosis. Potential contributors to metformin-associated lactic acidosis (MALA) include decreased renal function and decreased metabolic clearance resulting in accumulation of metformin. Formerly, NC OCME Toxicology relied on scene evidence and/or medical history to indicate a potential metformin-related case followed by send-out analysis. Starting in late 2024, our non-routine Drug Screen-Comprehensive detects metformin, with a semi-quantitative area count, determining if send-out confirmation is needed. Because metformin is widely prescribed, we have observed several presumptive positive cases since updating our workflow. Many of the confirmed cases have significant natural disease complications, including renal failure, lactic acidosis, and potentially euglycemic diabetic ketoacidosis (eDKA) associated with elevated metformin concentrations.

Objectives: Discussion about postmortem metformin cases in North Carolina, ranging from therapeutic to toxic, with a detailed exploration of the natural disease surrounding these deaths. Toxicologists will benefit in understanding our testing scheme and how we are relying on more than one testing paradigm to help provide information to the pathologists due to the unique clinical presentation of metformin-related complications.

Methods: A manual search of the laboratory management system uncovered 11 cases from 2023 to current where metformin was confirmed and reported. When possible, the vitreous urea nitrogen and creatinine results were included to help elucidate renal function. If applicable, hospital records may demonstrate acidosis at or near the time of death. These cases will be presented by cause and manner of death with the discussion including medical findings, toxicological findings, and pathologists' determination.

Results: The ages of the decedents (n=11) range from 34-67 with the following manner of death categories: natural (3), suicide (1), undetermined (4), and pending (3). The metformin concentrations in blood range from 0.22 to 85 mg/L, depicting therapeutic (<2 mg/L) to potentially toxic/lethal amounts (>5 mg/L presents an increased chance of acidosis). Urea nitrogen and creatinine concentrations range from normal to elevated postmortem levels, indicating possible renal disease or failure.

Age	Details	Metformin (mg/L)	Urea Nitrogen (mg/dL)	Creatinine (mg/dL)	Cause of Death
54	DM, HTN, alcoholism, obesity	0.34 (aorta)	13	0.3	Paroxetine, Citalopram, Ethanol Toxicity
45	DM II, hypercholesterolemia, GERD	1.9 (iliac)	38	1.1	Complications of DM, obesity contributing
47	DM, HTN, in emergency room with shortness of breath, increase creatinine, seizures	32 (serum)	108	4.5	Metformin Toxicity

Discussion: There are many commonalities between these cases, most cases had history of diabetes and had findings of kidney disease during examination. Only one case did not mention a history of diabetes and was determined to be suicidal in manner. Three case examples in the table reflect similarities in history and circumstances with differing cause/manner of death. The data spans from therapeutic metformin, with normal postmortem urea nitrogen and creatinine levels, to metformin toxicity with elevated electrolytes (possibly MALA). Because of the overlapping complexities with natural disease and exclusion from routine toxicological screening workflows, metformin overdose deaths may be rarely pursued and therefore underrepresented in the literature.

Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

P-245

Fatal Fentanyl-Associated Mixed-Drug Intoxication in a 46-Year-Old Woman with Psychiatric Comorbidities: Illicit Fentanyl Exposure in the Context of Prescribed Psychiatric Polypharmacy

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Abstract

Introduction: Fatal intoxications involving fentanyl are frequently complicated by the presence of multiple central nervous system depressants and psychoactive medications. While most published polydrug fentanyl fatalities involve co-intoxication with stimulants, heroin or alcohol, cases illustrating the convergence of illicitly sourced fentanyl with prescribed psychiatric polypharmacy stay underrepresented in the literature. This case involves a 46-year-old woman with a reported history of bipolar disorder and depression. No history of opioid prescription or documented substance use disorder was identified in the available records. According to family members, she had a pattern of mixing medications. On the day of death, she reportedly ate, fell asleep and was later heard snoring by her family, a finding often described in opioid related respiratory depression. Emergency medical services were contacted when she became unresponsive, and she was pronounced dead upon paramedic arrival.

Objectives: To describe a fatal mixed drug intoxication involving illicitly sourced fentanyl, tramadol, benzodiazepine exposure, antidepressants, and sedating antihistamines in a patient with psychiatric comorbidities and to evaluate the contribution of polydrug exposure to fatal respiratory and central nervous system depression.

Methods: Postmortem cardiac blood was analyzed using enzyme-linked immunosorbent assay, liquid chromatography/time-of-flight mass spectrometry, and liquid chromatography tandem mass spectrometry. Toxicological interpretation was performed in the context of the investigative history and reported behavioral circumstances preceding death.

Results: Toxicological analysis of cardiac blood identified fentanyl at 23 ng/mL and norfentanyl at 12 ng/mL. 4-ANPP, a fentanyl precursor associated with clandestine synthesis and a metabolite, was also detected qualitatively, suggesting illicitly manufactured fentanyl as the source of exposure. Additional findings included tramadol at 1000 ng/mL, clonazepam at 7.1 ng/mL, 7-aminoclonazepam at 28 ng/mL, duloxetine at 750 ng/mL, hydroxybupropion at 1500 ng/mL, hydroxyzine at 220 ng/mL, and caffeine. The fentanyl concentration in cardiac blood should be interpreted with thoughtfulness, given the well-documented potential for postmortem redistribution. However, when considered alongside the circumstances of death and concurrent exposure to multiple CNS depressants, the findings are consistent with fentanyl as a central contributor to the fatal intoxication. Tramadol was present above typical steady-state therapeutic concentrations, while hydroxyzine and clonazepam-related findings further supported sedative co-exposure. Duloxetine and hydroxybupropion were detected at concentrations consistent with recent use, though postmortem redistribution may have influenced the measured values.

Discussion: The circumstances of death, including sudden unresponsiveness after sleep and reported snoring, are consistent with opioid-induced respiratory depression. The toxicological profile supports a fatal mixed drug intoxication in which fentanyl played a central role, with additive or synergistic contributions from tramadol, clonazepam exposure, hydroxyzine, and antidepressant medications. The detection of norfentanyl and 4-ANPP supports fentanyl exposure from an illicit source, a finding of particular forensic relevance in a decedent with no documented opioid prescriptions. The concurrent detection of multiple prescribed psychoactive substances highlights the complexity of postmortem interpretation in individuals with psychiatric comorbidities and reported medication mixing. Postmortem redistribution, especially from cardiac blood, represents a significant limitation in the quantitative interpretation of all reported concentrations. This case emphasizes the forensic importance of evaluating fentanyl fatalities within a broader polydrug context and illustrates the underrecognized intersection between illicit fentanyl exposure and prescribed psychiatric polypharmacy.

Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

P-246

Prevalence and Forensic Interpretation of Toxicology Findings in Pediatric Mortality

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Abstract

Introduction: Interpretation of toxicological findings in pediatric death investigations presents unique challenges, particularly in distinguishing causative exposures from incidental drug detections. While toxicological analysis is frequently performed, the extent to which detected substances contribute to death in young children remains low but not negligible.

Objectives: The objective of this project is to retrospectively review pediatric toxicology data over the last ten years in Jefferson County, AL, and evaluate trends and potential diagnostic pitfalls.

Methods: A retrospective review was conducted of pediatric deaths (ages 0-10 years) over a 10-year period (n = 314) in Jefferson County, AL. Cases were categorized into three groups: (1) deaths directly attributable to toxicological causes, (2) deaths with positive toxicology findings not determined to be causal or contributory, and (3) deaths without identified toxicologic involvement or data. Demographic data, substances detected, and manner of death were evaluated.

Results: Of the 314 cases, toxicological analysis was performed on 283 cases (90%). Of the tested cases, a toxicological cause of death was found in 11 cases (3.9%), while 22 cases (7.8%) demonstrated the presence of drugs or toxic substances that were not considered causal or contributory. The remaining 250 cases (79.6% of total cases) had no drugs detected. Opioids, particularly methadone and fentanyl, were the most frequently identified substances in toxicological deaths. Incidental toxicology findings typically included benzodiazepines or morphine derivatives which, in many cases, were administered at the hospital during life-saving measures. Toxicological deaths were more likely to involve acute exposures and were predominantly classified as undetermined in manner. In contrast, non-causal toxicology findings occurred across a range of manners of death but were mostly accidental or homicide cases.

Discussion: Toxicological causes of death in children under age ten are relatively uncommon in Jefferson County, AL; however, the presence of detectable substances is more frequent and may complicate forensic interpretation. The identification of opioids in both causative and incidental contexts underscores the importance of careful interpretation of toxicology results, particularly in the setting of low-level detections. These findings highlight the need for contextual evaluation of toxicological data to avoid misattribution of cause of death in pediatric cases.

Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

P-247

Evaluation of the Impact of Methamphetamine-Induced Rhabdomyolysis on Postmortem Redistribution

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Abstract

Introduction: In Japan, methamphetamine (MA) is the most widely used illicit drug, and the forensic interpretation of its blood concentrations in fatal intoxications is critically important. Postmortem redistribution (PMR) is a major factor complicating the interpretation of postmortem drug concentrations, and MA is considered to be prone to PMR. In general, higher drug concentrations in cardiac blood than in peripheral blood (C/P > 1) are considered a characteristic finding of PMR. As femoral blood is relatively less affected by postmortem changes, it is often regarded as the preferred specimen for toxicological interpretation. However, in practice, cases showing higher drug concentrations in femoral than in cardiac blood (C/P < 1) are also observed. A possible explanation is PMR from skeletal muscle surrounding the femoral vein into femoral blood. Rhabdomyolysis is characterized by skeletal muscle injury resulting in the release of intracellular components into the blood circulation, which cause electrolyte imbalance and acute kidney injury and is frequently observed in MA users. Here, we investigated the association between rhabdomyolysis and the C/P ratio in MA-related deaths.

Objectives: We examined the hypothesis that skeletal muscle injury due to MA-induced rhabdomyolysis elevates MA concentrations in femoral blood, resulting in a C/P < 1.

Methods: A total of 11 cases in which MA was quantified in both femoral and cardiac blood were selected. Femoral blood and cardiac blood collected from the right atrium were obtained during autopsy and stored at –20 °C until analysis. Blood samples were extracted using a Micro Volume QuEChERS kit (Shimadzu). Quantitative analysis of MA was performed using an ultra-high-performance liquid chromatograph coupled with a triple quadrupole tandem mass spectrometer (LCMS8060NX, Shimadzu). Urinary myoglobin (Mb) levels were measured as a marker of rhabdomyolysis.

Results: MA concentration ranges in femoral and cardiac blood were 0.084-131 and 0.15-46 μg/mL, respectively. The median C/P ratio was 1.6 with a range of 0.4-1.9. Three cases had a C/P ratio < 1 (0.4, 0.5, 0.8) and showed elevated Mb levels (5,360–17,200). Whereas a case with a C/P ratio of 1.9 also exhibited a markedly elevated Mb level (14,000). Among cases with a C/P < 1, one had a postmortem interval (PMI) of 24–48 h, while the other two had PMIs ≥72 h.

Discussion: In these postmortem cases, a clear association between rhabdomyolysis (elevated Mb levels) and the C/P ratio could not be demonstrated due to multiple confounding factors affecting PMR (e.g., PMI, route of administration, and dose). However, the present findings suggest that PMR may also occur in peripheral blood under certain conditions, and that interpretation based solely on femoral blood concentrations may lead to misclassification of the cause of death. Accurate toxicological interpretation therefore requires blood analysis from multiple sampling sites. Furthermore, to more precisely evaluate the effects of rhabdomyolysis, we are conducting experimental studies using an animal model, and the latest findings will also be presented.

Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

P-248

Quantitative Measurement of Carbon Monoxide Using the HemoCD Assay: Differential Correlations with %COHb in Human Blood and Myocardial Tissue

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Abstract

Introduction: Carbon monoxide (CO) is one of the leading causes of fatal intoxication in various settings, including fires and suicides. The heart is considered particularly susceptible to CO due to its high oxygen demand and the abundance of myoglobin, which has a high affinity for CO. Cardiovascular complications have been reported in survivors of CO poisoning, including cardiomyopathy and arrhythmias, suggesting direct cardiotoxic effects of CO. The severity of CO poisoning is primarily assessed using blood carboxyhemoglobin saturation (%COHb), which, although a useful indicator of exposure, may not fully reflect myocardial CO burden. In fact, cardiac dysfunction has been reported irrespective of %COHb levels. Therefore, evaluation of tissue CO concentration is essential for understanding CO-induced cardiotoxicity; however, quantitative analysis of CO in human myocardial tissue remain limited.

Objectives: To directly quantify CO concentrations in human blood and myocardial tissue using hemoCD-P, an artificial hemoglobin reagent with high CO affinity, and to evaluate their association with %COHb.

Methods: Human blood (n = 22; %COHb 1 - 82) and myocardial tissue samples (n = 42; %COHb 1–83) collected at autopsy were analyzed. CO concentrations were measured using a modified hemoCD assay based on the method reported by Mao et al. Analytical validity of the assay was evaluated using blood samples with experimentally adjusted %COHb levels; the assay showed excellent linearity (%COHb 0–90, $R^2 = 0.996$) and a strong correlation with gas chromatography measurements ($R^2 = 0.978$). Reproducibility of myocardial CO measurements was confirmed with a median coefficient of variation (CV) of 6.0%. Myocardial samples were classified into three groups according to %COHb for group comparisons: control (<5, n = 10), moderate exposure (23–46, n = 7), and severe exposure (≥50, n = 25), with %COHb 50 defined as the conventional lethal threshold. Linear regression analysis was used to evaluate the relationship between CO concentration and %COHb. Normality of myocardial CO concentrations within each group was assessed using the Shapiro–Wilk test. Pairwise comparisons among groups were performed using Welch's t-test with Holm correction for multiple comparisons ($p < 0.01$).

Results: In blood, CO concentration exhibited a strong correlation with %COHb ($R^2 = 0.93$; $y = 3.7x + 14.2$). In contrast, myocardial CO concentration demonstrated a weaker correlation with %COHb ($R^2 = 0.57$). Myocardial CO concentrations were significantly higher in the moderate (51.6 ± 16.5 pmol/mg) and severe (62.7 ± 19.5 pmol/mg) groups than in controls (21.7 ± 5.4 pmol/mg), adjusted $p = 0.0025$ and $p < 0.001$, respectively, whereas no significant difference was observed between the moderate and severe groups, adjusted $p = 0.157$.

Discussion: These findings indicate that myocardial CO concentration increases with CO exposure but demonstrates a substantially weaker correlation with %COHb than blood CO concentration. Notably, myocardial CO concentrations exceeded approximately 40 pmol/mg in 94% of exposure cases regardless of %COHb, suggesting that fatal CO intoxication may be associated with myocardial CO concentrations above this level.

Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

P-249

Postmortem Brain Carbon Monoxide Concentrations in Two Fatal Carbon Monoxide Poisoning Cases with Decreased Blood COHb Saturation After Oxygen Therapy

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Abstract

Introduction: In carbon monoxide (CO) poisoning, blood carboxyhemoglobin (COHb) saturation is widely used to assess exposure and support diagnosis. However, post-treatment COHb saturation does not necessarily correlate with neurological severity or outcome and may not accurately reflect residual CO in tissues. We previously reported that CO concentrations in human postmortem brain tissue were approximately 20–30 pmol/mg in controls and 30–50 pmol/mg in fatal CO poisoning cases. In this study, we quantified postmortem brain CO concentrations in two fire-related fatal CO poisoning cases in which blood COHb saturation was high on hospital arrival but low at autopsy.

Methods: Two patients were transported from a fire scene and died after treatment. Neither underwent hyperbaric oxygen therapy. In Case 1, blood COHb saturation was 52.5% on hospital arrival and 2.9% at autopsy; the patient survived for approximately 11 hours after return of spontaneous circulation (ROSC). In Case 2, blood COHb saturation was 52.9% on hospital arrival and 1.8% at autopsy; the patient survived for approximately 47 hours after ROSC. At autopsy, samples were collected from eight brain regions: frontal cortex, frontal white matter, putamen, globus pallidus, internal capsule, caudate nucleus, temporal cortex, and temporal white matter. Brain tissue CO concentrations were measured using the hemoCD assay and compared with our previously reported control and CO poisoning groups.

Results: In Case 1, brain tissue CO concentrations ranged from 29.3 to 46.9 pmol/mg, with relatively high values in the temporal cortex, internal capsule, and frontal cortex. In Case 2, concentrations ranged from 17.1 to 23.9 pmol/mg and were lower overall than in Case 1. Compared with the previously reported groups, Case 1 values were generally within or near the range of the CO poisoning group, whereas Case 2 values were generally within or near the control range. Thus, despite similarly high blood COHb saturation on hospital arrival and low values at autopsy, the degree of residual CO in the brain differed markedly between the two cases. This difference may be related to survival time after ROSC. Regional differences in CO distribution were also observed, suggesting that intracerebral CO clearance after treatment may not be uniform. These findings are consistent with experimental data showing that CO can remain in tissues, including the brain, even after blood COHb saturation has decreased.

Discussion: These findings suggest that, even in fatal CO poisoning cases with reduced blood COHb saturation after treatment, the extent of residual CO in brain tissue may vary between cases, and that differences in survival time after ROSC may contribute to this variation.

Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

P-250

Still Present, Still Lethal: Methamphetamine-Positive Deaths in a Changing Drug Landscape

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Abstract

Introduction: Methamphetamine continues to contribute substantially to drug-related fatalities; however, recent public health and forensic toxicology focus has largely centered on synthetic opioids, particularly fentanyl, and the emergence of novel psychoactive substances (NPS). While these represent critical and evolving threats, this emphasis may obscure the sustained impact of more traditionally established stimulants. Methamphetamine remains widely available and frequently encountered in postmortem toxicology in both single- and polysubstance deaths.

Objective: A retrospective review of methamphetamine-positive fatalities in Travis County, Texas (TX), and surrounding areas from 2021 to 2025 was conducted to characterize regional trends, demographic patterns, and toxicological findings.

Method: Postmortem cases submitted to the Travis County Medical Examiner for toxicology testing from 2021-2025, were queried to identify the total number of methamphetamine-positive deaths reported during this timeframe. Demographic (sex, race, age) and toxicological (methamphetamine concentrations and polysubstance trends) information was gathered for all methamphetamine-related fatalities and evaluated annually and in totality. All cases analyzed using LC-MS/MS, liquid/liquid extraction, with an LOQ of 10ng/mL.

Results: Methamphetamine was identified in 2017 deaths from 2021-2025, accounting for 17.6% of postmortem cases (n=11446), with peak positivity occurring in 2023 (20.6%). Statistically significant ($p<0.05$) differences in the number of methamphetamine-positive deaths were only observed between 2021 (n=350) and 2023 (n=501), and 2023 (n=501) and 2025 (n=317). Methamphetamine-related fatalities primarily occurred in white males in the early 40's. Concentrations ranged from 10-87000 ng/mL with mean ± SD (median) concentrations of 1253 ± 3444 (430) ng/mL. Of the 2017 methamphetamine-positive cases, 79% were ruled an accident (n=1596), followed by homicide (8.8%; n=178), and suicide (6.7%; n=135). Over half of the methamphetamine-positive deaths were attributed to methamphetamine-related drug toxicities (58.4%; n=1178) and primarily ruled accidental deaths (n=1164). Interestingly, the cause of death in 11 of the homicide cases was also partially attributed to the combined effects of methamphetamine toxicity. Mean ± SD (median) concentrations observed in methamphetamine-related toxicities (1459 ± 3786; 480 ng/mL) were statistically significantly greater ($p<0.05$) compared to incidental findings (955 ± 2440; 390 ng/mL). Although polydrug use was prevalent in most fatal drug toxicities, methamphetamine was attributed as the sole cause of death in 435 of these cases (36.9%). Fentanyl and cocaine were the most common co-occurring substances found with methamphetamine.

Discussion: Methamphetamine remained a significant contributor to mortality in Travis County and surrounding areas from 2021-2025, accounting for a substantial proportion of postmortem toxicology cases. Although peak positivity was observed in 2023, methamphetamine presence remained consistently elevated, emphasizing its sustained impact within a broader period of shifting drug trends. While polysubstance use was common, particularly with fentanyl and cocaine, more than one-third of fatal drug toxicities were attributed solely to methamphetamine toxicity, highlighting its independent lethality. The predominance of accidental deaths and high frequency of methamphetamine-related drug toxicities reinforces its role in unintentional overdose deaths. Additionally, the presence of methamphetamine in a subset of homicide cases, where it contributed to the cause of death, underscores its relevance in medicolegal death investigations. Despite increasing attention towards synthetic opioids and NPS, methamphetamine remains a critical and potentially underemphasized driver of drug-related fatalities, warranting continued surveillance and analytical focus.

Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

P-251

Withdrawn

Withdrawn

P-252

Summary Statistics of Post-Mortem Blood Concentrations of Pharmaceuticals and Metabolites: An Analysis of Portuguese Data Over a Two-Year Period

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Abstract

Introduction: In Portugal, it has been possible to collect and statistically process national toxicological data thanks to the ongoing efforts to standardize the technologies, materials and methods used at the three operational units of the Forensic Chemistry and Toxicology Service (Lisbon, Oporto and Coimbra) of the National Institute of Legal Medicine and Forensic Sciences. In recent years, more than 200 substances have been routinely analyzed whenever pharmaceuticals analysis is requested. Of these, 65% act on the central nervous system, 20% act on the cardiovascular system and the remaining 15% belong to other pharmacological groups, according to the classification of the Portuguese Therapeutic Index. From the set of substances analyzed, we present the statistical results for the 100 pharmaceuticals and metabolites most frequently found in post-mortem (PM) blood, corresponding to more than 50 PM concentrations for each substance.

Objectives: To identify the most frequently pharmaceuticals detected in Portugal, and to collect and statistically analyze the concentrations obtained in order to provide a data-driven interpretation of pharmaceutical-related deaths.

Methods: Concentration data were obtained from post-mortem blood samples (femoral blood in 98% of cases), collected during autopsies and stored in polypropylene tubes containing 1% NaF at low temperatures (-20 to -10 °C) prior to analysis. The blood samples underwent routine quantitative analysis to detect around 200 substances, using a comprehensive method available in all three laboratories. Sample preparation involved precipitating proteins from whole blood using acetonitrile (-20°C), followed by centrifugation. The resulting supernatant extracts were analyzed on ultra-high-performance liquid chromatographs coupled with triple quadrupole linear ion trap mass spectrometers (Sciex UHPLC-QTRAP-MS® 6500+) in Multiple Reaction Monitoring (MRM) mode, with two transitions for each compound.

Results: The concentration distributions of 100 pharmaceuticals and metabolites found in post-mortem blood were statistically characterized based on an extensive database of 14160 autopsy cases investigated over a two-year period in the portuguese forensic laboratories of the National Institute of Legal Medicine and Forensic Sciences (2023–2024). The systematic approach adopted in this study, introduced by Jones and Holmgren and often referred to as the “all-cause-of-death approach”, utilizes PM concentrations accumulated during the case analysis, without any pre-selection of cause of death. The proportion of men was 62%, with a

median age of 65 years, compared with 37% of women, with a median age of 72 years. In 1% of cases, this information is unknown or undetermined.

Discussion: The mean, median and upper percentiles (90th, 95th and 97.5th) pharmaceutical concentrations were determined. The median PM concentration provides an indication of the "normal" PM concentration level, whilst the upper percentiles indicate possible levels of overdose. This is the most comprehensive data analysis to date on drugs and their metabolites in the PM blood in Portugal. Whenever possible, our results were compared with previously published PM concentrations in blood.

Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

P-253

Cocaine and Death Due to Aortic Dissection: A Case Report.

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Abstract

Introduction: Aortic dissection is a rare but serious cause of sudden death linked to cocaine use. While most cocaine-related deaths result from acute toxicity, overdose, myocardial infarction, or arrhythmias, aortic dissection represents a smaller yet significant share. Cardiovascular mortality in cocaine users is more than twice that of non-users, accounting for 3.9–5.4 % of sudden deaths in forensic studies.

Objectives: To report the case of a deceased woman due to aortic dissection and the presence of cocaine. Case Report: A 55-year-old female, weighing 72kg and measuring 1.64m, was found at her home; She had likely been dead for about 24 hours. The autopsy revealed an aortic dissection extending from the aortic valve annulus to above the diaphragm; the cause of death was cardiac tamponade due to aortic dissection.

Methods: The blood was analyzed by solid-phase extraction, next to detection, confirmation, and quantification of drugs using GC-MS, with analysis conducted at the Iquique Medico Legal Service (SML) Laboratory. Alcohol analysis was performed using a dual-column GC-FID-HS system at Copiapó SML Laboratory. The autopsy was performed in accordance with the SML's standardized procedures.

Results:

Toxicology:

Sample	Cocaine	Benzoylecgonine	Ethanol	Methanol	Acetone	Isopropanol
Femoral blood	1.35 mcg/ml	Detected	N/D	N/D	N/D	N/D
Bladder wash	Detected	Detected	N/D	N/D	N/D	N/D

N/D: Not Detected.

Thanatochemistry:

Sample	Troponin T Ultra Sensible	CK-MB	NT-proBNP
Femoral blood	4308.0 ng/l	549.00 ng/ml	348 pg/ml
Vitreous humor	351.5 ng/l	3.67 ng/ml	49 pg/ml

Discussion: Toxicology: Cocaine was quantified in a femoral blood sample; this blood level correlates with possible intoxication, as the postmortem concentration falls within lethal levels (0.9–21 mcg/ml), and benzoylecgonine was detected in femoral blood and bladder wash. No ethanol, methanol, acetone, or isopropanol were detected in the samples, ruling out their influence on the death.

Thanatochemistry: Currently, there are no universally accepted reference values for ultra-sensitive troponin T, CK-MB, and NT-proBNP in postmortem blood, due to variations associated with the postmortem interval, the sampling site, and the degree of decomposition. In forensic practice, the upper limits established in healthy living populations (14 ng/l, 25U/l, and 220pg/ml, respectively) are used as a guide, interpreting them with caution based on the postmortem context and available literature. In this case, the three cardiac markers showed concentrations significantly higher than these values.

Pathology: The autopsy revealed a tension hemopericardium of 500 cc, indicating cardiac tamponade; an extensive thoracic aortic dissection was observed, extending from the aortic valve annulus to above the diaphragm, leaving the thoracic aorta reduced to two large, extensive cavities containing clotted blood. The heart showed mixed cardiomyopathy, with features of left ventricular dilation and hypertrophy and a preserved valvular apparatus.

Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

P-254

Fatal Intoxication Involving the Novel Synthetic Opioids SR-17018 and Methiodone: A Case Report

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Abstract

Introduction: Novel Synthetic opioids (NSOs) play an increasingly important role within the illegal drug market. These drugs are associated with potential risks that are frequently challenging to anticipate, and which exhibit variable potency levels that are not yet fully characterized. Methiodone (IC-26) and SR-17018 (5,6-dichlor-desmethylchlorphine) are emerging opioids for which there is limited toxicity data and no established clinical use. SR-17018 belongs to the newly emerging group of “orphine” opioids, and thus is structurally related to brorphine and cychlorphine. Here, the body of a 63-year-old man was discovered in his apartment, along with resealable bags containing powders and tablets. At autopsy, unspecific indicators of intoxication were found.

Objectives: The aim of this case report is to present the first postmortem case involving the novel synthetic opioids SR-17018 in combination with methiodone including their concentrations in biological specimens as well as the in vitro potency of SR-17018 at the μ-opioid receptor (MOR). Furthermore, particular challenges of toxicological interpretation in the context of the absence of established postmortem reference concentrations for SR-17018 will be discussed.

Methods: Femoral blood, heart blood, liver, bile, kidney, gastric contents and postmortem urine were collected during autopsy and subjected to toxicological analysis. Screening for drugs of abuse and volatile substances including ethanol was performed using high performance liquid chromatography-diode array detector (HPLC-DAD) and gas chromatography-flame ionization Detector (GC-FID). Screening results were confirmed in femoral blood and urine by liquid chromatography-tandem mass spectrometry (LC-MS/MS). SR-17018 was quantified in postmortem samples using a standard addition method. Femoral blood and urine samples were analysed through external matrix calibration. Tissue distribution and postmortem redistribution of SR-17018 were evaluated. Receptor activation was evaluated using an HTRF®-assay measuring G protein signaling as increase in fluorescence upon MOR activation.

Results: The concentrations of SR-17018 in femoral blood and urine were 15 ng/mL and 22 ng/mL, respectively. Methiodone concentrations of 560 ng/mL and 1200 ng/mL were detected in femoral blood and urine, respectively. Other findings in femoral blood and urine were cychlorphine (0.1 ng/mL) and 3,4-EtMC (3,4-ethylenemethcathinone) (< 1 ng/mL). In vitro assay data establishes SR-17018 as MOR agonist (E_{max}=110 % of fentanyl) with an EC_{50}-value of 227 nM. Besides those substances, diphenhydramine, fluoxetine and norfluoxetine could be detected in femoral blood, but in a therapeutic range.

Discussion: The case involves a multiple drug intoxication by methiodone and SR-17018. The potency of the substance SR-17018 is situated between methiodone and fentanyl. The elevated concentration of methiodone in combination with SR-17018 and cychlorphine can be regarded as fatal. This case contributes to the forensic knowledge on the new synthetic opioid SR-17018 and emphasizes the relevance of NSOs in general toxicological screening strategies.

Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

P-255

Testing Validity of the Standardized Field Sobriety Test (SFST) to Detect Presence of and Impairment by Cannabis and/or Alcohol: A Randomized Clinical Trial

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Abstract

Introduction: The Standardized Field Sobriety Test (SFST) is widely used to detect substance-related impairment at the roadside. While its validity for alcohol is well established, its ability to detect cannabis-related impairment and reflect functional driving impairment remains unclear.

Objectives: This study assessed: (1) classification accuracy of the SFST following alcohol and/or cannabis use; (2) dose–response relationships between breath alcohol concentration (BrAC), blood delta-9-tetrahydrocannabinol (THC), and SFST performance; and (3) the association between SFST performance and simulated driving outcomes after alcohol and/or cannabis use.

Methods: A within-subjects, double-blind, placebo-controlled randomized trial was conducted in 25 regular cannabis users aged 19–29 years. Participants completed four conditions: placebo, alcohol (target BrAC 0.08%), cannabis (12.5% THC), and alcohol+cannabis. SFST performance and simulated driving outcomes (standard deviation of lateral position, speed variability, mean speed, maximum speed, and braking latency) were assessed following substance administration. Classification accuracy compared SFST pass/fail outcomes against per se impairment thresholds (BrAC ≥ 0.08%, blood THC ≥ 5 ng/mL). Correlational analyses of change scores, threshold-based comparisons, and mixed-effects models were also conducted.

Results: Total SFST scores were higher under alcohol and alcohol+cannabis compared to placebo (both $p<.001$), whereas cannabis did not differ from placebo. Classification accuracy varied across substances. In the cannabis condition, sensitivity was 25.0% and specificity was 84.6%. In the alcohol condition, sensitivity was 58.8% and specificity was 62.5%. In the alcohol+cannabis condition, sensitivity reached 83.3% and specificity decreased to 36.8%, depending on impairment definition.

SFST scores were not associated with blood THC in the cannabis condition ($p=.30$). In the alcohol+cannabis condition, SFST scores were associated with BrAC ($p=.015$) but not THC ($p=.36$). Analyses using continuous biomarker levels did not identify consistent associations with SFST performance, whereas grouping by per se thresholds showed differences in SFST scores across conditions. SFST scores were not associated with simulated driving outcomes. Participants who failed the SFST did not differ in driving performance from those who passed.

Discussion: Within this controlled laboratory study, SFST performance varied across substances, showing greater sensitivity to alcohol-related effects than to THC-defined impairment. This pattern is consistent with original development of the SFST as a measure of alcohol-related psychomotor impairment.

Lack of consistent associations between SFST performance and THC levels, together with the absence of relationships with simulated driving outcomes, indicates that SFST scores may not reflect cannabis-related impairment or functional driving performance under these conditions. These findings suggest that the observable signs assessed by the SFST do not necessarily correspond to underlying impairment in driving-related functions.

Differences between correlational and threshold-based analyses further indicate that the relationship between biomarkers and behavioral performance depends on the analytical approach. Continuous biomarker levels did not map consistently onto SFST performance, whereas threshold-based groupings revealed differences across conditions, indicating that biological measures of substance exposure may not directly map onto observable impairment.

Together, these findings indicate that SFST performance reflects general behavioral effects of substance use but may not provide a direct measure of functional impairment. These results support interpreting SFST outcomes within a broader assessment framework, particularly in the context of cannabis use.

Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

P-256

Crabs, Old Bay, and Controlled Substances: A Ten-Year Retrospective Analysis of Impaired Maryland Drivers

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Abstract

Introduction: The Toxicology Section at Maryland State Police, Forensic Sciences Division (MSP-FSD) was established in 2009 to perform toxicology testing for driving under the influence of alcohol (DUI) and drug (DUID) investigations. From 2015 to 2025, the state of Maryland and MSP-FSD faced numerous events, including the opioid epidemic, the COVID-19 pandemic, and the state legalization of marijuana in 2023, that have changed operations. Additionally, the laboratory has expanded from one, centralized laboratory to include two satellite locations in the western and eastern regions of Maryland.

Objectives: A retrospective analysis of 2015 to 2025 blood alcohol and blood drug cases was conducted to review any change, frequency, concentrations, and polydrug trends.

Methods: MSP-FSD is accredited by ANSI National Accreditation Board (ANAB) and licensed through the Maryland Department of Health. The laboratory is accredited for quantitative analysis of blood alcohol via Headspace GC-FID, and the qualitative analysis of drugs via ELISA, GC-MS, and LC-MS/MS. Per state statute, only DRE officers can request drug analyses. Currently, the Toxicology Section analyzes 28 drugs and metabolites, but this landscape is constantly evolving with the creation of new methods and updates to instrumentation. Blood alcohol and blood drug cases collected in approved blood kits and tested from 2015 to 2025 were reviewed.

Results: From 2015-2025, there were a total of 6,854 and 5,551 cases analyzed for blood alcohol and blood drug, respectively. From 2015-2023, the number of case submissions remained relatively consistent around 1,000 cases per year, but a decline in case submissions was observed in 2023, which has since continued. For blood alcohol cases, an average of 66% were positive for alcohol, with 59% at or above the legal limit (0.08 g/100 mL) and of these, 93% were above 0.1 g/100 mL. The highest number of alcohol positive cases (81%) was observed in 2025. Additionally, approximately 8% of all cases contained alcohol concentrations between 0.01-0.07 g/100 mL. Approximately one-third of all submitted alcohol cases were negative for alcohol, but only 28% of negative alcohol cases were re-submitted for drug testing.

69% of requested drug cases were positive for one or more drugs that can cause impairment. The top analytes were THC/metabolites, fentanyl, alprazolam, methadone, morphine and benzoylecgonine. The most frequently detected drug(s) for 2015-2025, with the exception of 2019-2021, were THC/metabolites. From 2019-2021, the most frequently detected was fentanyl.

Discussion: Maryland has seen an impact in impaired driving cases following the COVID-19 pandemic, marijuana legalization, and changing technologies. More than half of the blood alcohol positive cases were above the 0.08 g/100 mL legal limit with the majority falling between 0.10-0.29 g/100 mL. To date, the highest BAC result was 0.500 g/100 mL. Drug trends have stayed consistent, with THC/metabolites and fentanyl being the most frequently identified, but new drugs are constantly emerging. Understanding current and past drug trends can help inform communities and continue advancing forensic toxicology endeavors.

Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

P-257

Roadside Surveys of Drinking and Driving in Cameroon

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Abstract

Objectives: This study aimed to assess the prevalence of drinking and driving on roadways in urban areas and highways in Cameroon and to determine the associations between drinking and driving and socio-demographic factors.

Methods: A cross-sectional study of motor vehicle drivers was performed on Fridays, Saturdays, and Sundays between May and September 2020 with three driver groups: (1) random motor vehicle drivers (including riders) on major highways, (2) drivers recruited at car stations in Yaounde, and (3) at fuel stations in Douala. Alcohol was measured using breathalyzers, and a questionnaire collected socio-demographic data.

Results: In total, 2402 motor vehicle drivers were asked to participate in the study and 1701 (70.8%) gave informed consent. The vast majority (98.6%) were men. Drivers aged 30-39 years constituted the largest age group on highways and in Yaounde, whereas 18-29 years was the largest age group in Douala. The highest prevalence of alcohol was observed among drivers in Yaounde, which included mainly clandestine taxi car drivers and motorcycle taxi riders, where about 30% had blood alcohol concentrations (BAC) above the legal limit of 0.08%. The proportion with BACs above the legal limit was about 6% among the drivers in Douala, which included mainly motor-cycle taxi riders, and about 4% among drivers on highways.

Discussion: The findings indicate that drinking and driving is a major traffic safety problem on Cameroonian public roads, especially among motorcycle taxi riders and clandestine taxi drivers in towns, which represent the major mass transportation means in the country. Drinking and driving education and legislation should be better developed and enforced in order to reduce the number road traffic crashes.

Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

P-258

Unbiased Screening of DUID Cases for Gamma-Hydroxybutyric Acid (GHB)

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Abstract

Introduction: The Office of the Chief Medical Examiner for the City and County of San Francisco, assesses all driving casework for GHB as part of a comprehensive forensic panel of drugs and drug metabolites using a validated LC-MS/MS method. While GHB is produced endogenously at low concentrations in various tissues, it can also be taken as a drug. GHB can be used medically for the treatment of narcolepsy (Xyrem), however it has a reputation for use in drug facilitated crime and is used recreationally as a "club drug", often in combination with Methamphetamine. GHB is classified as a central nervous system depressant and is taken recreationally for its euphoric and sedative effects, which can contribute to driving impairment.

Objectives: The objective is to provide information on GHB detection in DUID cases from July 2021 to December 2025, in addition to other drug classes detected, and demographic information on drivers.

Methods: Blood and urine samples were screened using previously validated LC-MS/MS methods to detect and quantitate GHB, in addition to a comprehensive panel of drugs and drug metabolites. GHB detections ≥ 5000 ng/mL were considered positive.

Results: Out of the driving cases screened for the 4.5 year period (n =2492), 10 were positive for GHB. Of these cases, GHB was detected in 9 blood samples (7000-110,000 ng/mL) and 1 urine sample (1,100,000 ng/mL). No alcohol was detected in any of the GHB positive cases. Six of the drivers were male while four of the drivers were female. All of the 10 DUID cases positive for GHB included one or more additional drug detections. In addition to GHB, the following drug classes were detected: stimulants, cannabinoids, opiates, benzodiazepines, antidepressants, hallucinogens and anti-epileptic drugs. Methamphetamine and/or amphetamine was detected in 9/10 GHB-positive cases.

Discussion: Unbiased screening for GHB in DUID cases in San Francisco identified 10 detections in driving cases from 2021-2025. While the prevalence of GHB use in drivers is relatively low, it is advantageous to screen cases for GHB as part of a drug panel due to the potential impact of the drug on driving impairment. These results align with a recent study in the same jurisdiction and period, where 75% of GHB-related fatal accidental overdoses would have been missed if targeted testing was applied as there was no indication of GHB use during the death investigation.

Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

P-259

Five-Year Retrospective Analysis of Alcohol and Drug Involvement in Motor Vehicle-Related Fatalities in Jefferson County, Alabama

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Abstract

Introduction: The Jefferson County Coroner/Medical Examiner's Office (JCCMEO), based in Birmingham, AL, was responsible for completing 5,878 death investigations over the past 5 years. Of these, 47% were accidental deaths, with 22% (602) of these from motor vehicle accidents (MVAs). The effects of substance abuse in MVAs are less apparent than in a drug overdose population but equally impactful on public health. For this reason, a five-year retrospective analysis was conducted on JCCMEO MVA cases from 2021-2025 to elucidate their statistics.

Objectives: The objective of this retro-analysis was to evaluate data collected by the University of Alabama at Birmingham forensic toxicology laboratory to (1) identify trends and characterize patterns within MVAs over five years, (2) assess relationships between motor vehicle fatalities and prevalence of alcohol and drugs, and (3) evaluate the impact of demographic and substance abuse factors.

Methods: Forensic toxicological analysis was conducted on post-mortem samples (blood/urine/bile/tissues) collected by the JCCMEO. Methods included volatiles analysis by HS-GC-FID, drugs of abuse screening by EMIT or ELISA, and confirmation and quantitation by GC/MS. Results were compiled and shared with JCCMEO medical examiners using a collaborative interactive database. The toxicological results used to aid in cause-of-death (COD) determination were evaluated for the retro-analysis presented herein.

Results: In the previous five years, MVAs accounted for approximately 10% of all assumed cases and 22% of accidental deaths. These decedents were 64% drivers, 11% passengers, and 23% pedestrians or cyclists. The gender demographics were predominantly male (72%).

An average of 35% of cases had alcohol present (≥ 0.01 g/dL) with 21% having alcohol as a contributing factor to COD. Additionally, an average of 30% of cases had "drugs recommended for toxicological investigations of drug-impaired driving and motor vehicle fatalities" detected.1 9% had these drugs as a contributing factor to COD, with CNS stimulants being the most abundant class and methamphetamine being the most abundant drug.

Of the 387 cases where the decedent was the driver, 216 (56%) involved ethanol and/or the drugs. 31% of drivers had blood alcohol levels over the state legal limit (0.08 g/dL). Decedents aged between 25-35 were the most common drivers at 21% and the most likely to be driving over the legal limit at 24% of cases.

Along with ethanol-involved cases, drugs of abuse were detected in 28% of MVAs where the decedent was the driver. The most abundant drug class was CNS stimulants at 43%, followed by narcotic analgesics at 29% and CNS depressants at 26%. The top five most prevalent drugs were amphetamine (<0.02 - >5.0 mg/L), methamphetamine (0.056 - >1 mg/L), cocaine (0.025 – 0.69), fentanyl (<0.0025 - 0.232 mg/L), and diphenhydramine (< 0.025 - 0.5 mg/L).

Discussion: From this retrospective analysis, it is evident that motor vehicle fatalities are heavily impacted by the presence of alcohol and psychoactive drugs with over half of the motor vehicle fatalities in Jefferson County being influenced by substance abuse. With this type of analysis and other comparable studies both nationally and internationally, stakeholders can maintain awareness of the impact of using such psychoactive and pharmacological agents.

References

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Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

P-260

Drug Detection Trends in Umbilical Cord and Meconium and the Impact of Enzymatic Hydrolysis in Cord Analysis

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Abstract

Introduction: In utero drug exposure remains a critical public health concern with potential impacts on neonatal development. Because maternal substance use is frequently underreported, analytical testing provides a more objective assessment of exposure. Umbilical cord tissue (UCT) and meconium (MEC) are the two most commonly utilized matrices for neonatal drug detection. In MEC testing, hydrolysis is widely recognized as a necessary step to improve detection of drug conjugates and is routinely implemented in clinical laboratories. In contrast, although UCT has been increasingly adopted as an alternative matrix, the role of hydrolysis in UCT testing is less well established and not uniformly applied across laboratories. In this study, drug detection in both UCT and MEC was evaluated to characterize prevalence trends, and enzymatic hydrolysis was further assessed for its impact on analyte detection in UCT specimens.

Objectives: UCT and MEC specimens were analyzed to assess drug positivity rates, concentrations, and prevalence trends; a subset of UCT specimens was further analyzed with and without enzymatic hydrolysis to evaluate its impact on analyte detection.

Methods: De-identified, non-paired remnant clinical UCT (n=123) and MEC (n=84) specimens were extracted by automated solid phase extraction (SPE) and analyzed via liquid chromatography-tandem mass spectrometry (LC-MS/MS) using a SCIEX QTRAP 6500 system. All analytes were monitored using at least 2 transitions and 1 ion ratio. One transition was used for each internal standard (ISTD). Validation followed CLSI, ASB, and internal protocols. A comprehensive semi-quantitative 71-drug panel was employed, encompassing 8 drug classes, including opioids (23), antidepressants (13), benzodiazepines (11), stimulants (7), sedatives/dissociative anesthetics (5), anticonvulsants (2), antipsychotics (2), barbiturates (2), alongside specific analytes such as meprobamate, dextromethorphan, cotinine, mitragynine, acetyl fentanyl, and xylazine. Analyte cutoffs were standardized across matrices, with a subset requiring higher thresholds in MEC.

Results: Overall positivity rates with hydrolysis were similar between MEC (56%) and UCT (51%). Cotinine was the most frequent analyte in both matrices (~39%), followed by opioids (MEC: 18%; UCT: 12%). Within the opioids class, norbuprenorphine has the highest rate of detection ranging from 33 ng/g to 711 ng/g (average 348 ng/g) in MEC and 2 ng/g to 25 ng/g (average 11 ng/g) in UCT. Antidepressants were consistently detected (MEC: 9%; UCT: 11%), while muscle relaxants, xylazine, acetyl fentanyl, and mitragynine were not observed. In a subset of positive UCT specimens (n=28), hydrolysis significantly improved opioid detection. Without hydrolysis, 75% of buprenorphine (3 of 4), 100% of naloxone (1 of 1), and 100% of oxymorphone (2 of 2) positives would have been missed. Hydrolysis also increased measured concentrations by ~130% for cotinine and quetiapine, and ~270% for morphine.

Discussion: In this study, both UCT and MEC demonstrated comparable overall positivity rates, with matrix-dependent differences by drug class. Drug concentrations were generally lower in UCT, highlighting the need for sensitive analytical approaches. Incorporation of enzymatic hydrolysis in UCT testing improved detection of conjugated analytes and reduced false negatives, particularly for buprenorphine, naloxone and oxymorphone. The impact of hydrolysis across additional drug classes warrants further evaluation in broader cohorts.

Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

Conflict of Interest

Authors are current employees of Quest Diagnostics & have received salaries and may own stock.

P-261

Exploring Human Bone Specimens as an Alternative Biological Matrix for Drug Screening Using Gas Chromatography-Mass Spectrometry (GC-MS): Potentialities and Limitations

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Abstract

Introduction: Postmortem toxicological analysis is essential in drug-related death investigations. However, traditional matrices like blood and urine are often unavailable in cases involving skeletonization, prompting the emergence of bone as a potential alternative matrix. Its application remains limited due to its structural complexity, including variability in bone type, which may influence drug incorporation and distribution. Although prior research has confirmed the presence of drugs in bone, significant gaps remain, including the lack of validated methods and insufficient data for comparison with conventional matrices.

Objectives: This study aimed to develop, optimize, and apply an analytical workflow for the screening of selected drugs in bone using gas chromatography–mass spectrometry (GC-MS).

Methods: Authentic blank human femur specimens collected from the Southeast Texas Applied Forensic Science (STAFS) facility. Samples were pre-processed through cleaning, maceration, chemical washing, drying, and homogenization into powdered bone. Extraction was performed using an ultrasound-assisted solid–liquid extraction with methanol. After the addition of methanol, an aliquot of internal standards was added, followed by centrifugation, filtration, and pentafluoropropionic anhydride (PFPA) derivatization. Instrumental analysis was conducted using an Agilent 6890N GC-MS system in splitless and full scan and SIM modes (three ions monitored per analyte). Method validation was performed and limit of detection (LOD), selectivity, carryover, recovery, and post-processing stability were assessed. The validated method was applied to nine authentic bone specimens with known drug exposure histories.

Results: A mass of 100 mg of bone was selected as the optimal mass for extraction and preparation of positive controls using no more than 50 µL of standard working solutions. PFPA was selected as the derivatization reagent to improve the GC-MS detection of amphetamine-type compounds. The optimal condition for extraction included horizontal agitation and sonication for 60 minutes. Alprazolam, and the pair amphetamine and ketamine were excluded from the scope due to poor detection and failure to meet identification criteria, respectively. LODs were 500 ng/g for MDA, MDMA, and diazepam, and 2500 ng/g for methamphetamine, PCP, cocaine, and fentanyl. Endogenous interferences were observed but did not exceed detection thresholds, and no carryover or external interferences were detected. Recoveries were generally above 50% but varied by source, indicating matrix source-dependent effects. Analytes remained detectable after storage in the autosampler for up to 48 hours. All authentic specimens tested negative for the target drugs.

Discussion: These findings demonstrate that GC-MS can be used for drug screening in human bone, but significant limitations remain. Bone's complex, mineralized structure may restrict solvent penetration and analyte recovery. Analytical challenges, including limited GC-MS sensitivity, ion-ratio variability and matrix interferences, were observed. A structured decision workflow was developed to improve applicability by prioritizing peak shape, retention time, and signal-to-noise ratio and ion-ratio evaluation, considering the challenges outlined above. The absence of detectable drugs in authentic specimens suggests additional limitations, including variability in drug incorporation, postmortem degradation, uneven skeletal distribution, and analyte loss during pre-processing. Overall, these results emphasize the need for further research to improve analytical methods and establish reliable interpretive frameworks for bone toxicology.

Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

P-262

Evaluation of the Cross-Reactivity of the Narcotrace™ 8-Drug Panel Oral Fluid Device for Cannabinoid and Semi-Synthetic Cannabinoid Compounds

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Abstract

Introduction: Point-of-care testing (POCT) is a common format for clinical products, allowing for on-site testing and rapid results. Collection of oral fluid (OF) is less invasive than urine, making it an attractive alternative for screening in both clinical and nonclinical settings, particularly in roadside investigations, where contemporaneous sample collection would be beneficial. The presence of Δ9-tetrahydrocannabinol (Δ9-THC) in OF demonstrates high specificity and selectivity when used as a biomarker of exposure. POCT devices for Δ9-THC in OF are marketed for various forensic and clinical applications, however, cross-reactivity with other phyto-, semi-synthetic, and synthetic cannabinoids is often not well characterized.

The Narcotrace™ 8 Drug Panel (Narcotrace™ Panel) immunoassay is utilized for the forensic identification of d-amphetamine (AMP), morphine (OPI), d-methamphetamine/3,4-methylenedioxymethamphetamine (MET/MDMA), Δ9-THC, cocaine (COC), fentanyl (FEN), oxazepam (BZO) and ketamine (KET). This device is designed to detect drugs in saliva, sweat, powders, gummies, or on surfaces and is intended for use by law enforcement, forensic examiners, or employers.

Objectives: To evaluate the sensitivity of the Narcotrace™ Panel for the detection of Δ9-THC at the reported 25 ng/mL cutoff, and assess the cross reactivity of ten cannabinoid analogues in OF.

Methods: Narcotrace™ Panels were evaluated according to the manufacturer instructions for reactivity with Δ9-THC using negative OF (UTAK SMx Oral Fluid) as the matrix at concentrations of 500, 200, 100, 50, 25, 10, and 0 ng/mL. The samples were prepared at a volume of 200 µL in OF. Each sample, and controls of Type 1 water and 5% methanol in OF, were aliquoted at a volume of 75 µL onto the collection device and analyzed according to manufacturer instructions. The following ten cannabinoids were evaluated for cross-reactivity; cannabidiol (CBD), Δ8-THC, Δ10-THC, Δ6a,10a-tetrahydrocannabinol (Δ6a,10a-THC), hexahydrocannabinol (HHC), Δ9-tetrahydrocannabiphorol (Δ9-THCP), Δ9-tetrahydrocannabutol (Δ9-THCB), Δ9-tetrahydrocannabihexol (Δ9-THCH), Δ9-tetrahydrocannabivarin (Δ9-THCV), and Δ9-tetrahydrocannabinol acetate (Δ9-THC-O). Initial testing was performed at 500 ng/mL in OF. If cross-reactivity was observed, serial dilutions (200, 100, 50, 25, and 10 ng/mL) were tested sequentially until negative results were observed. Results were evaluated by a minimum of 3 individuals to limit individual bias.

Results: All drug-free matrices were confirmed to be negative. Interpretation of Δ9-THC and additional cannabinoid results varied among observers. In the first trial for Δ9-THC observers agreed on the lowest detected value of 100 ng/mL, however, in a repeat trial the lowest detected value was 25 ng/mL. Cross-reactivity was observed for all THC analogues at 500 ng/mL except Δ9-THCP. Below this concentration, Δ9-THCB and Δ9-THC-O were detected unanimously at 200 and 100 ng/mL respectively.

Discussion: There was inter-day and inter-observer variability in interpretation of results. Cross-reactivity was observed for all THC analogues evaluated with the exception of Δ9-THCP. The Narcotrace™ devices, though simple to use, had limited analogue cross-reactivity at the reported 25 ng/mL Δ9-THC cutoff and inconsistencies with observer interpretation of Δ9-THC OF samples.

Disclosure

Yes, I, or a member of my immediate family, has a financial interest to disclose.

Conflict of Interest

Yes, third-party research support. Funding Grant Number: [15PNIJ-23-GG-01421-COAP].

P-263

Mimicking the Matrix: Early Development of a Synthetic Serum Blank for Toxicological Applications

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Abstract

Introduction: Reliable toxicological analysis depends on the availability of appropriate reference materials that closely replicate the biological matrix of authentic samples. Serum-based matrices are widely used in clinical and forensic toxicology; however, sourcing consistent and well-characterised blank serum materials remains challenging due to variability, ethical considerations, and potential presence of endogenous substances and drugs of abuse that may interfere with analytical methods. The development of a synthetic serum blank that accurately mimics the physicochemical properties of human serum could provide a reproducible and controlled alternative for method development, validation, and quality assurance.

Objectives: This study aims to design and develop a synthetic serum blank reference material that closely resembles human serum in composition and analytical behaviour. The goal is to evaluate its suitability for use in forensic and clinical toxicology applications, particularly in supporting accurate qualitative and quantitative analyses.

Methods: A formulation strategy is being developed to replicate key components of human serum, including protein content, lipid composition, ionic strength, and pH. Candidate formulations will be assessed for their physicochemical properties and matrix effects using commonly employed toxicological screening and confirmatory techniques (e.g., LC–MS/MS and GC–MS). Initial evaluation will focus on reproducibility, absence of interfering substances, and comparability to authentic serum in terms of analyte recovery and signal response.

Results: Work is ongoing. Currently, several formulations are available for synthetic serum but are typically designed for specific attributes of the desired application. These do not necessarily represent native serum and therefore may not provide representative results. The solution being designed is based on true pooled serum component concentrations and includes not just proteins and minerals, but also small molecule components such as amino acids, steroid-type compounds and metabolic waste byproduct compounds, present at lower concentrations which can impact analytical performance on drug recovery. The synthetic serum should be as close a match to true serum to get valid quality control results and be able to catch possible method bias.

Discussion: The development of a synthetic serum blank has the potential to address key limitations associated with biological reference materials, including variability, supply constraints and native background contamination. A well-characterised synthetic matrix could enhance method standardisation and improve inter-laboratory comparability in toxicological analyses. Ongoing work will determine the extent to which such a material can replicate matrix effects observed in authentic samples and identify areas for further refinement. Future studies can include expanded analyte panels and collaborative evaluation across laboratories.

Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

P-264

Extraction of NSAIDs from Horse Urine Using QuEChERS

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Abstract

Introduction: Non-steroidal anti-inflammatory drugs (NSAIDs) are utilized in human and veterinary medicine to reduce inflammation, pain, and fever. Due to their ability to mask injury and reduce lameness, NSAIDs are highly regulated in horse racing. However, horse urine is a highly complex matrix, complicating extraction of analytes for toxicological analysis. QuEChERS (Quick, Easy, Cheap, Rugged, and Safe) sample preparation, which includes a dispersive solid-phase extraction (dSPE) cleanup step, can be used to extract a wide array of analytes from complex matrices while requiring less time and resources than traditional methods such as liquid-liquid and solid-phase extraction.

Objectives: Develop a QuEChERS sample preparation method for the analysis of NSAIDs in authentic horse urine, with a comparative evaluation of conventional dSPE and SpinFiltr® cleanup formats.

Methods: Fifteen NSAIDs from various classes were spiked at 100 ng/mL in triplicate and extracted from authentic urine collected from a 9-year old mare. Enzymatically hydrolyzed urine samples were added to 15-mL QuEChERS tubes containing 400 mg of MgSO4 and 100 mg of sodium acetate. Ethyl acetate was added to each sample prior to mixing and centrifugation. The supernatant was transferred to either dSPE 2 mL centrifuge tubes or SpinFiltr® microcentrifuge tubes, each containing 150 mg of MgSO4 and 50 mg of C18. Samples were thoroughly mixed and centrifuged. Supernatant or pull-through was transferred to clean test tubes, evaporated under nitrogen at 40°C, and reconstituted for analysis by LC-MS/MS. Analysis was performed using a Shimadzu Nexera LC-30AD with MS-8050. NSAIDs were separated using a SelectraCore® C18 (2.1 x 100 mm, 2.7 µm) column with an accompanying guard column. The aqueous mobile phase was 0.1% ammonium hydroxide in water, and the organic mobile phase was methanol. A nine-minute gradient was established with all analytes eluting within 7 minutes.

Results: Calibration curves were established for all analytes over a range of 5 to 1,000 ng/mL. Traditional dSPE cleanup yielded an average extraction efficiency of 97%. Performing the same dSPE cleanup using the SpinFiltr® format resulted in an average extraction efficiency of 94%. The lowest observed recovery across all analytes and extraction methods was 74%. Relative standard deviation was below 20% for all analytes regardless of the dSPE format used. Overall, dSPE cleanup removed markedly more matrix components than other extraction techniques tested.

Discussion: Multiple classes of NSAIDs were efficiently extracted from horse urine using a QuEChERS approach. The cleanup can be performed using traditional dSPE format or simplified by using dSPE in SpinFiltr® format, which eliminates the need to transfer the supernatant after the dSPE step and streamlines sample preparation. NSAIDs have varied chemistries but are generally weakly acidic in nature. Using ammonium hydroxide as a mobile phase additive allowed analytes to be ionically charged thereby improving chromatographic performance on a reverse-phase column. The high-purity superficially porous silica that makes up the stationary phase of SelectraCore® performed consistently and reliably even under these alkaline conditions.

Disclosure

Yes, I, or a member of my immediate family, has a financial interest to disclose.

Conflict of Interest

Salary

P-265

Analyzing the Impact of Centrifugation and Extraction Aids on Oral Fluid Sample Recovery

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Abstract

Introduction: Oral fluid is a relatively simple matrix to collect and prepare; however, the collection kits can cause issues downstream. Quantisal™ collection kits consist of an applicator with attached sponge, a tube with buffer solution, and a cap. The total volume for testing should be 4 mL (1 mL of oral fluid and 3 mL of buffer); however, it is difficult to recover the full amount. There are different techniques to manipulate the sponge and improve oral fluid recovery. Popular techniques include manual compression, centrifugation, and the use of an accessory to assist with manual compression. This work also tested and compared an oral fluid extraction accessory (OFEA) accompanied with a centrifugation step to previously mentioned techniques. This study compared these four extraction techniques by examining volume recovery and analyte recovery.

Objectives: Demonstrate the advantages of incorporating a centrifugation step and the use of extraction aides to sample preparation to determine its impacts on volume and analyte recovery when performing analysis of drugs of abuse and (DoA) in oral fluids by LC-MS/MS.

Methods: Thirty-one common DoA compounds were separated by LC-MS/MS using a Biphenyl column under gradient conditions using water and methanol both containing 0.1% formic acid, with a total cycle time of 7 minutes. Samples were prepared in synthetic oral fluid and combined with Quantisal™ buffer. Four recovery techniques were compared and tested in triplicate: manual compression, centrifugation, manual compression with an accessory, and centrifugation with OFEA. Samples were prepared using a salt-assisted liquid-liquid extraction (SALLE).

Results: Four techniques were evaluated by examining total volume removed and analyte recovery. Volume recovery was tested by pouring the solution into a graduated cylinder and recording the total volume recovered. When using centrifugation with the OFEA, an additional 500 µL was collected compared to the other techniques. Analyte recovery was compared by spiking a known concentration, 50 ng/mL, into each of the samples. Samples were evaluated using a calibration curve prepared without using the kits. Peak areas increased for all analytes when using the centrifugation and centrifugation with the OFEA. Accuracy and precision were assessed by analyzing if the results fell within +/- 15% of the target as well as comparing %RSD values. Manual compression with the accessory and centrifugation with the OFEA showed the best accuracy and precision with comparable results to each other. A study was also conducted to assess efficiency of each of the techniques. Four samples were prepared by each technique, and the time was then multiplied to give an indication of time for 12, 48, and 96 samples. When preparing 96 samples, the centrifugation with the OFEA is the fastest technique by up to 32 minutes.

Discussion: Four techniques were compared to extract liquid from the sponge of oral fluid collection kits. Through examining volume and analyte recovery, it was clear that the centrifugation step paired with the oral fluid extraction accessory showed the best results for total volume recovered as well as increased analyte recovery when analyzed quantitatively compared to the other techniques.

Disclosure

Yes, I, or a member of my immediate family, has a financial interest to disclose.

Conflict of Interest

Salary/Consultant

P-266

Case of Consistency: Oral Fluid as a Tool to Monitor Mental Health Medications in Clinical Settings

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Abstract

Introduction: Oral fluid testing provides unique advantages that should be considered by clinical providers. It is an easy and dignified collection method, medications measured in the oral fluid are generally reflective of what is actively circulating in the bloodstream, and it offers a detection window more aligned with prescribing practices. This case study demonstrates the effectiveness of therapeutic drug testing using oral fluid to monitor four medications (hydroxyzine, 9-hydroxyrisperidone, mirtazapine, and trazodone) in one patient over 10 months of sampling.

Objectives: To determine the effectiveness of oral fluid toxicology testing for therapeutic drug monitoring when paired with robust medication dosing and sample collection information.

Methods: Twenty oral fluid samples were collected for medically necessary testing from a single patient over a 10-month treatment period using the Quantisal (TM) oral fluid collection device. Samples were collected twice per month during this timeframe. Samples underwent direct-to-definitive LC-MS/MS testing for a panel of prescribed medications and illegal substances. Medication dose, dosing frequency, time of last dose, and time of sample collection, were recorded for each test. Data analysis was conducted under IRB-2024-359 approval from Western Michigan University. Prescribed medications, doses, and dosing frequencies remained unchanged across all collections (hydroxyzine 24 mg PRN; 9-hydroxyrisperidone 12 mg QD; mirtazapine 45 mg QHS; trazodone 150 mg PRN).

Results: All 20 samples were positive for the four prescribed medications, with no additional substances detected. Mean oral fluid concentrations were as follows: hydroxyzine 5.4 ng/mL (range 3.3–18.1 ng/mL; mean 2.8 hours post-dose), 9-hydroxyrisperidone 6.5 ng/mL (3.9–15.7 ng/mL; 17.6 hours post-dose), mirtazapine 3.4 ng/mL (1.5–14.7 ng/mL; 17.6 hours post-dose), and trazodone 5.2 ng/mL (3.1–19.8 ng/mL; 17.6 hours post-dose). Dosing times relative to sample collection were consistent, with hydroxyzine typically taken 1–3 hours prior and the remaining medications taken 17–18 hours prior. Most quantitative oral fluid results clustered between the 25th and 75th percentiles, indicating consistent concentrations. The highest values for each medication occurred in the first sample and correlated with shorter dosing-to-collection intervals.

Discussion: This dataset is notable for repeated, near-identical testing in a single individual over time. Quantitative oral fluid concentrations were observationally consistent and reflective of a patient who was compliant with what was being prescribed as well as how it was being prescribed. Medication presence or absence does not always indicate compliance with prescribing regimens; matrix considerations and quantitation should be evaluated. Urine testing does not discern recent from historical use and blood testing can be burdensome for most medication monitoring. Oral fluid sampling is rapid, convenient, and generally reflective of what is in the bloodstream at the time of sampling. Understanding quantitative oral fluid results requires

accurate dosing and timing information and should be applied longitudinally at the individual level rather than across populations. Toxicology results are not a silver bullet and should be used within the totality of a clinical circumstance to help providers ask better questions. This case supports the potential use of quantitative oral fluid toxicology for therapeutic drug monitoring.

Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

P-267

Evaluation of THC Acid A as a Marker for External Contamination for the Analysis of Capillary Blood from the Finger Pulp

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Abstract

Introduction: Recent research (Bantle et al., DTA 2026) demonstrated that THC concentrations in dried blood spots (DBS) from capillary blood significantly deviate from results in venous blood (VB), while the metabolite THC-COOH showed good agreement. It is hypothesized that this deviation results from cannabis plant material contamination on finger pulps. Furthermore, it is hypothesized that THCA-A is a marker for such contamination, because THCA-A is abundant in raw plant material but is thermally decarboxylated during cannabis consumption.

Objectives: This study aims to assess the potential of THCA-A as a "contamination marker" and to test the efficacy of different sample site pre-cleaning methods for removal of residual cannabis. Furthermore, we aimed to include the metabolite 11-OH-THC in the comparison of capillary and VB samples to evaluate capillary blood collection as a simple alternative to VB collection.

Methods: Samples were collected and analyzed using a protocol adapted from Bantle et al. Briefly, 20 µL of capillary blood were collected from n = 96 recreational consumers (SCRIPT pilot trial, University of Bern) using volumetric DBS devices (DBSV, Protzek, Germany). Extraction involved incubating the DBS with 50 µL dimethyl sulfoxide, followed by extraction with 1 mL methanol. The LC-MS/MS method was extended to include 11-OH-THC and THCA-A. Decontamination was investigated by taking swabs with water-soaked DBSV devices (Protzek) from the index and middle finger after joint preparation. Samples were collected in duplicate, either without decontamination or after decontamination with alcohol-free tissues, soap and water, or isopropanol. The swab samples were analyzed using the same protocol as for capillary blood samples.

Results: The analysis yielded 46 quantitative results for THCA-A and THC, as well as 45 for 11-OH-THC and THC-COOH, in both capillary and VB. The mean DBS-to-VB concentration ratios for THCA-A, THC, 11-OH-THC, and THC-COOH were 74.0 ± 88.5, 10.1 ± 23.8, 1.4 ± 0.7, and 1.4 ± 0.5, respectively. Swab samples taken either without decontamination or after decontamination with alcohol-free tissues, soap and water, or isopropanol showed THCA-A concentrations of 144, 94, 37, and 28 ng/mL and THC concentrations of 3155, 820, 236, and 111 ng/mL, respectively.

Discussion: The concentration ratios of the metabolites 11-OH-THC and THC-COOH confirm the feasibility of capillary blood analysis. However, the mean THC ratio indicates significant deviations between capillary blood and VB. The high mean THCA-A ratio suggests that elevated THC concentrations in capillary blood originate from external contamination. Alternative

explanations, such as accumulation in fingertip adipose tissue, are improbable as venous THCA-A concentrations are very low even at high THC concentrations. Additionally, due to the similar structure, accumulation of THC-COOH would also be expected if tissue distribution caused deviations. While THCA-A showed potential as a contamination marker, additional experiments are needed as all capillary blood samples, including samples with a THC DBS-to-VB ratio close to one, showed elevated THCA-A concentrations. Decontamination studies showed that isopropanol is the most efficient cleansing agent; however, significant analyte amounts were still detected. Consequently, optimisation of sample site pre-cleaning is essential to validate capillary blood analysis for forensic applications.

Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

P-268

Socioeconomic Stratification in Alcohol Consumption During the Pandemic: Evidence from Wastewater-Based Epidemiology and Predictive Decision Modelling

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Abstract

Introduction: Wastewater-based epidemiology (WBE) provides objective, anonymized, and near real-time estimates of community substance use, complementing self-reported surveys and sales statistics. During the COVID-19 pandemic, changes in mobility, economic conditions, and social behavior may have affected alcohol consumption differently across socioeconomic groups. However, population-level evidence capturing such differences with high spatial and temporal resolution remains limited, particularly in middle-income settings.

Objectives: This study investigated changes in alcohol consumption across districts in Türkiye before, during, and after the COVID-19 pandemic, with emphasis on socioeconomic differences. It also assessed the use of a CatBoost-based machine learning model to predict WBE-derived alcohol consumption patterns and identify key drivers of variation.

Methods: This study used data from a nationwide WBE monitoring program conducted in Türkiye between 2019 and 2023. Influent wastewater samples were collected from 43 wastewater treatment plants, generating more than 4,000 data points. Sampling was performed four times per year, corresponding to spring, summer, autumn, and winter. In each seasonal campaign, all 43 plants were sampled concurrently for seven consecutive days using 24-hour composite influent samples. Ethyl sulfate (EtS), the alcohol consumption biomarker, was quantified using a validated dilute-and-inject LC-MS/MS method. Population-level alcohol consumption was back-calculated from EtS concentrations using daily flow data, a literature-based correction factor, and population normalization. Districts were classified according to the SEGE-2025 socioeconomic development index. COVID status was defined as the study period category, including pre-COVID, COVID, and post-COVID periods. Although broad national coverage was achieved, the sampling design was not geographically or urban–rural balanced; therefore, the dataset represents monitored wastewater catchments rather than an equally distributed national sample. CatBoost regression was used because of its ability to model nonlinear relationships, interactions, and categorical district-level variables, and feature importance was evaluated by permutation analysis.

Results: The model showed strong predictive performance, with a mean absolute percentage error of approximately 3–4%, corresponding to 96–97% accuracy. District was the strongest predictor, followed by COVID status and season, whereas socioeconomic level had low direct importance when district-specific effects were included. Alcohol consumption dynamics differed across socioeconomic strata. Consumption in disadvantaged districts remained relatively stable. Middle socioeconomic districts showed a decline during the COVID

period, which became more pronounced post-COVID. Affluent districts showed increased consumption during the pandemic, followed by a marked post-pandemic decline.

Discussion: These findings indicate that pandemic-related changes in alcohol consumption were not homogeneous but were mediated by district-level and socioeconomic context. Micro-geographical variation appeared to explain behavioral patterns better than broad crisis indicators alone. The CatBoost model supported the interpretation of complex WBE datasets and the identification of nonlinear behavioral patterns. As this study focused on alcohol consumption, future research should integrate illicit drug biomarkers to provide a broader picture of substance use dynamics. Overall, combining WBE with predictive decision modelling may support targeted monitoring, prioritization of high-variation districts, and more efficient allocation of public health resources.

Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

P-269

Assessment of Second-Hand Nicotine Exposure via Cross-Sectional Biomonitoring

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Abstract

Introduction: Accurate assessment of passive tobacco smoke exposure in occupational settings is challenging, particularly at low exposure levels. Self-report may underestimate exposure, whereas urinary nicotine biomarkers provide objective evidence of recent tobacco smoke exposure. Urinary pH may also affect ionization, extraction efficiency, and analytical behavior of nicotine-related compounds.

Objectives: This study aimed to develop and validate a sensitive LC-MS/MS method for urinary nicotine biomarkers, evaluate passive tobacco smoke exposure in a nominally smoke-free industrial workplace, and assess pH-related effects on method robustness.

Methods: A cross-sectional on-site study was conducted at a large food-production facility in Türkiye. Participants completed a questionnaire on tobacco smoke exposure during the previous seven days at home, in vehicles, public places, and the workplace, and provided mid-shift spot urine samples. Urinary pH was measured directly using a calibrated digital pH meter. Extraction was performed using ToxTube®; the extraction solution pH was 6.80. Urine matrices were adjusted to pH 3.13, 4.77, 5.42, and 6.80 to evaluate pH-related analytical effects. Urinary nicotine (NIC), cotinine (COT), and trans-3′-hydroxycotinine (3HC) were quantified using an LC-MS/MS method validated according to ANSI/ASB Standard 036. Cotinine-d3 was used as the internal standard. Dilution integrity was assessed using a 1:10 protocol; samples above 500 ng/mL were diluted with blank urine matrix, re-extracted, re-analysed, and corrected by the dilution factor. Participants reporting nicotine replacement therapy, including gum or patches, were excluded. To reduce smoking-status misclassification, questionnaire data were reviewed with urinary COT, and self-reported non-smokers with COT >100 ng/mL were excluded from the passive-exposure group.

Results: The method was linear from 0.5 to 500 ng/mL, with R^2 >0.99 and no significant matrix interferences. LOD/LOQ values were 0.13/0.41 ng/mL for NIC, 0.18/0.53 ng/mL for COT, and 0.11/0.34 ng/mL for 3HC. The final cohort included 42 employees: 28 passive-exposure non-smokers and 14 active smokers. NIC, COT, and 3HC were significantly higher in active smokers than passive-exposure non-smokers (all $p<0.001$). Median concentrations increased from 2.12 to 390.35 ng/mL for NIC, 2.46 to 843.50 ng/mL for COT, and 4.69 to 677.10 ng/mL for 3HC. Among passive-exposure non-smokers, biomarkers were more strongly associated with household exposure than workplace exposure duration. In pH testing, nicotine showed the greatest pH-dependent variability, including retention-time shifts and peak-shape changes, but analytical performance remained within acceptable limits; COT and 3HC remained comparatively stable.

Conclusions: Validated urinary biomonitoring distinguished passive exposure from active smoking in a smoke-free industrial setting. Combining LC-MS/MS biomarkers with questionnaire data improved interpretation beyond self-report. Cotinine was the most analytically robust biomarker for passive tobacco smoke exposure assessment.

Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

P-270

Rapid Enzymatic Hydrolysis for Fast Screening of New Psychoactive Substances in Urine Samples from Emergency Toxicology Cases

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Abstract

Introduction: The detection of new psychoactive substances (NPS) in clinical toxicology remains challenging due to extensive metabolism, often limiting the detectability of parent compounds in urine. The rapid and accurate identification of NPS is crucial in emergency toxicology, given their unknown nature and varying toxicities. Enzymatic hydrolysis is commonly employed to improve sensitivity for compounds undergoing phase II conjugation metabolism (glucuronidation and sulfation); however, conventional protocols are time-consuming and not suitable for emergency settings. Rapid hydrolysis approaches may overcome these limitations.

Objectives: To evaluate a rapid enzymatic hydrolysis protocol for urine samples from clinical emergency cases and compare its performance with a non-hydrolyzed approach for the detection of NPS and other drugs.

Methods: Six urine samples from different cases attended at an emergency department in Campinas-Brazil were analyzed using a rapid enzymatic hydrolysis protocol with β-glucuronidase (B-One®, Kura Biotech). Briefly, 250µL of urine were mixed with 50µL enzyme and 10µL internal standard followed by 5-minute incubation (room temperature) and without pH alteration. After addition of sodium tetraborate (300µL) and MTBE (1,200µL), samples were vortexed, centrifuged, frozen (–80°C, 10 min), the organic phase was collected, evaporated at 40°C, and reconstituted in 50µL of methanol. Analyses were performed using LC-MS/MS and LC-HRMS.

Results: Hydrolysis significantly improved the detection of compounds undergoing conjugation metabolism, consistent with the expected role of enzymatic deconjugation. In LC-MS/MS analysis, signal enhancement was observed across multiple classes, including an approximately 168-fold increase for 25B-NBOH, 6-fold for naloxone, 3-fold for 2,5-dimethoxy-4-bromoamphetamine (DOB), and 2-fold for bromazolam — all compounds known to form glucuronide or sulfate conjugates. Several compounds were detected only after hydrolysis, including metonitazene, 4-hydroxy-nitazene, N-pyrrolidino-4-hydroxy-nitazene (N-pyrrolidino-protonitazene metabolite), MDMB-4en-PINACA butanoic acid, 2C-B (25B-NBOH metabolite), and 25E-NBOH. LC-HRMS analysis confirmed these findings: 25B-NBOH increased approximately 236-fold, while DOB and bromazolam showed approximately 4-fold increases. N-pyrrolidino-protonitazene, 4-hydroxy-nitazene, and MDMB-4en-PINACA butanoic acid were confirmed exclusively after hydrolysis. Compound identification was supported by high-resolution MS/MS fragmentation, with diagnostic fragment ions consistent with reference spectra.

Discussion: Although the sample size of six cases is limited, the diversity of NPS and co-detected substances across these samples reflects the clinical reality of NPS intoxication, in which polydrug exposure is common and rarely involves a single compound. The proposed protocol achieves a total turnaround time of under 40 minutes per sample by integrating a rapid 5-minute enzymatic hydrolysis with streamlined extraction and analysis. This efficiency offers a significant advantage over conventional protocols that require at least one hour for the hydrolysis step alone. The substantial signal increases observed — attributable to the release of conjugated metabolites — together with the detection of analytes exclusively after hydrolysis, highlight the critical role of enzymatic deconjugation in urine analysis. The combination of LC-MS/MS with LC-HRMS provided complementary analytical capabilities, combining sensitivity with high-confidence identification, particularly relevant for structurally complex NPS such as N-benzyl-hydroxy-substituted phenethylamines, nitazenes, and synthetic cannabinoids. Overall, fast enzymatic hydrolysis using B-One® represents a practical and efficient strategy to enhance the detection and identification of NPS and other drugs in clinical and forensic toxicology settings.

Acknowledgments

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Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

P-271

Terbufos Intoxication in Humans and Animals: Toxicokinetics and Analytical Findings from Two High-Profile Taiwanese Cases

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Abstract

Introduction: Confirming intoxication by the highly toxic organophosphate terbufos presents a unique analytical challenge. Due to rapid systemic metabolism into active and inactive metabolites, detecting the parent compound in blood is frequently difficult.

Objectives: This presentation highlights two recent, high-profile forensic cases in Taiwan—an accidental human mass poisoning and a malicious animal poisoning—demonstrating the critical importance of analyzing gastric contents and residual food to confirm terbufos exposure.

Methods: Sample preparation was performed via methanol-induced protein precipitation. Biological specimens and environmental evidence were subsequently analyzed by LC-MS/MS (Waters Xevo TQ-S micro) and GC-MS (Thermo ISQ 7000). Liquid chromatography was achieved on a Phenomenex Biphenyl column (100 × 2.1 mm) with detection in multiple reaction monitoring (MRM) mode. Gas chromatography was performed using a Restek Rtx-5 column (30 m × 2.5 mm, 2.5 µm).

Results: The first case involves a tragic 2024 mass poisoning in Taitung, where terbufos-contaminated millet dumplings caused four fatalities and multiple ICU admissions. In 11 victims, RBC acetylcholinesterase levels (1–67) and blood terbufos concentrations (2.48–60.36 ng/mL) strongly correlated with clinical severity. Inactive metabolites (DEP, DETP, DEDTP) were quantified in all victims, whereas the active metabolite terbufos oxon sulfoxide was identified exclusively in the four deceased and one critical patient.

The second case (Yilan) involves malicious animal cruelty via a drone dropping terbufos-laced alfalfa pellets, killing an alpaca, a sika deer, and a pygmy goat. While GC-MS confirmed terbufos in the recovered pellets, animal blood and urine tested negative for the parent compound. However, gastrointestinal samples tested definitively positive for terbufos.

Discussion: Both investigations revealed a consistent toxicological pattern: systemic circulation samples predominantly exhibited metabolites, with the parent drug at low or undetectable levels. Conversely, gastrointestinal contents and recovered food vehicles yielded exceptionally high concentrations of the parent compound.

Due to rapid systemic metabolism, relying solely on blood analysis for terbufos may yield ambiguous results or false negatives. Securing and analyzing gastric contents and residual food is paramount for detecting the parent compound and unequivocally identifying the intoxication agent.

Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

P-272

Extraction of Tobacco-Specific Nitrosamines (TSNAs) from Human Urine Prior to UPLC-MS/MS Analysis

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Abstract

Introduction: TSNAs, or tobacco-specific nitrosamines, are carcinogens found in tobacco products, including e-cigarettes and smokeless tobacco. NNK (4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone), NNAL (4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol), and NNN (n-nitrosonornicotine) are the most analyzed TSNAs. In laboratory animal studies, NNK and its metabolite NNAL has been identified as a cause of lung cancer. Furthermore, NNN has been observed to induce esophageal cancer in these same animal models. Accurate quantification of TSNAs can be helpful to differentiate smokers from secondhand and third-hand contamination which can be challenging if the detection limits for the methods are low.

Objectives: This study demonstrates an optimized and streamlined extraction workflow for TSNAs in urine with high analyte recoveries, low matrix effect, and excellent reproducibility.

Methods: TSNAs were spiked at 200 pg/mL and extracted from non-hydrolyzed, non-smoker human donor urine only. Sample extractions were performed with a supported liquid extraction (SLE) protocol using ISOLUTE® SLE+ 1 mL cartridges and solid phase extraction (SPE) protocol using EVOLUTE® EXPRESS CX 30 mg 96 well plates. Extraction performance was evaluated by assessing analyte recovery, and matrix effect. The recovery is calculated as the ratio (%) of analyte peak areas between the pre-and post-spiked samples. The matrix effect is calculated by the ratio of peak areas between the post-spiked sample and the same analyte concentration spiked in the reconstitution solution. Calibration curves were constructed using concentrations ranging from 1.0 to 1000 pg/mL of synthetic urine (UriSub, pH 7.4) to avoid signal interferences. Limit of quantifications (LoQs) were determined from the calibration curves for each analyte by applying linear regression model. LC-MS/MS analysis was performed using a Shimadzu Nexera X2 UHPLC system coupled to a SCIEX 5500 MS system.

Results: Extraction recovery for TSNAs from SLE extraction protocol with ethyl acetate as elution solvent were 91.8%, 92.1%, and 80.7% with matrix effect of 0.82, 0.92, and 0.87 for NNAL, NNK, and NNN respectively. Extraction recovery for TSNAs from SPE protocol were 84.4%, 86.3%, and 85.9% with matrix effect of 1.07, 0.88, and 0.89 for NNAL, NNK, and NNN respectively. Analyte recoveries higher than 80% and matrix effect values within the range of 0.8 to 1.2 from both SLE and SPE methods show higher extraction efficiency with minimum matrix suppression or enhancement. Calibration curves for TSNAs spiked into UriSub showed good linearity with R2 values > 0.99 for both methods. LoQs obtained from SLE protocol were 7.0, 15.0, and 31.0 pg/mL and SPE protocol were 65.0, 33.0, and 28.0 pg/mL for NNAL, NNK, and NNN respectively.

Discussion: The load-wait-elute extraction procedure utilizing ISOLUTE® SLE+ offers a simple, fast, and automation compatible solution to extract TSNAs for LC-MS/MS analysis with high recovery and minimum matrix effect. On the other hand, SPE sample clean-up procedure provides an alternative method where the protocol can be fine-tuned to enrich a particular analyte for the detection and quantification at low-pico gram levels. The ability to quantify TSNAs at low pg/mL levels using either methods would allow the accurate monitoring of second-hand tobacco exposure and greatly facilitate toxicological studies.

Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

P-273

A Five-Year Comparison of Seized Drug and Toxicology Trends

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Abstract

Introduction: The interpretation of forensic toxicology findings occurs downstream of drug availability within a community. Seized drug evidence represents substances entering the illicit market, while toxicology results reflect confirmed biological exposure to those substances. Examining these datasets together provides an opportunity to evaluate the relationship between drug availability and observed toxicological findings. Despite this connection, seized drug and toxicology data are often evaluated independently within forensic laboratories. Integrating these datasets may provide additional insight into emerging drug threats, shifts in drug availability, and patterns of drug use within a region. Evaluating these trends collectively may also help laboratories anticipate future analytical needs and better contextualize toxicological findings observed in casework.

Objectives: The objective of this study was to evaluate trends in seized drug submissions and toxicology findings within a forensic laboratory over a five-year period and identify areas of convergence or divergence between the two datasets.

Methods: A retrospective review of laboratory data from January 2021 through December 2025 was conducted. Seized drug chemistry submissions and toxicology analytical results were extracted from the laboratory information management system. Submissions were categorized by major drug classes including opioids, stimulants, benzodiazepines, cannabinoids, and other emerging substances. Temporal trends in seized drug identifications were compared with the frequency of corresponding analytes detected in toxicology casework to identify changes in prevalence and determine whether emerging substances observed in seized drug evidence were subsequently reflected in toxicology findings.

Results: Across the five-year period, seized drug submissions were dominated by traditional drugs of abuse including methamphetamine, cocaine, and cannabis-related compounds, while additional trends were observed involving synthetic opioids, benzodiazepines, and other emerging substances. Toxicology findings demonstrated both similarities and differences when compared with seized drug data. In several instances, increases in seized drug identifications appeared to precede the detection of corresponding analytes in toxicology specimens, suggesting that seized drug evidence may provide an early indication of substances likely to appear in toxicology casework. Conversely, certain compounds frequently encountered in seized drug evidence were rarely observed in toxicology casework. Temporal comparisons also demonstrated fluctuations in the prevalence of specific drug classes across both datasets.

Discussion: Comparative evaluation of seized drug and toxicology datasets provides a broader perspective on regional drug trends and may enhance interpretation of toxicological findings. Similarities between the datasets may reflect consistent patterns of drug use within the community, while differences highlight the complex relationship between drug availability, patterns of use, pharmacology, and the likelihood of toxicological detection. Seized drug data may serve as an early indicator of substances entering the illicit market, whereas toxicology findings represent confirmed biological exposure. Because seized drug analysis is typically completed with a rapid turnaround time (average: 10 days) compared with toxicology testing (average: 30 days), these data may function as an early warning system for emerging substances that may warrant inclusion in toxicology screening or confirmation panels. Integrating information from both disciplines may improve situational awareness within forensic laboratories, support proactive method development, and assist laboratories in prioritizing analytical scope expansion as new substances emerge.

Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

P-274

Toxicological Assessment of Fentanyl, Xylazine, and BTMPS Through Zebrafish Developmental Model

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Abstract

Introduction: The illicit drug landscape has undergone significant changes over the past decade, shifting from plant-based drugs and opioids to synthetic substances such as fentanyl. Fentanyl has emerged as a major public health threat, and in recent years common adulterants of fentanyl, the veterinary tranquilizer xylazine and the UV stabilizer BTMPS (bis (2,2,6,6-tetramethyl-4-piperidyl)-sebacate), have been reported as additional concerns for public health and safety. Despite the increasing prevalence of these adulterants, limited data exist on developmental and toxicological impacts of these compounds, particularly when present in combination with fentanyl.

Objectives: This study employs FET (fish embryo acute toxicity) and MTC (maximum tolerated concentration) tests, along with morphological and physiological assessments to evaluate the individual and synergistic toxicity of fentanyl, xylazine, and BTMPS across multiple developmental stages of zebrafish (Danio rerio), providing insights into their individual and combined toxicological effects.

Methods: All procedures were conducted in accordance with OECD guidelines outlined in Test Guideline No. 236 and approved by the IACUC. Adult zebrafish (2 males:1 female) were placed in breeding tanks overnight. The following morning, the divider separating the sexes was removed, allowing spawning and subsequent egg fertilization to occur. At 2–3 hours post-fertilization (hpf), embryos were collected, sorted and rinsed with E3 embryo medium while the adult fish were returned to the holding tank. For the FET test, embryos were placed individually into 96-well plates, containing 100% E3 embryo medium (control) or drug solutions prepared in E3 embryo medium at six different concentrations ranging from 5–500 µM, selected based on published lethal dose ranges and pilot range-finding experiments. Exposure occurred from 2–3 hpf to 4 days post-fertilization (dpf). Lethal endpoints were defined at embryo coagulation, lack of somite formation, failure of tail detachment from the yolk sac, or lack of heartbeat. For the MTC test, the larvae that hatched from the FET test continued exposure between 4 dpf and 9 dpf. Endpoints included morphological deformities, lack of heartbeat, locomotor issues, delayed growth, and death.

Results: Fentanyl alone demonstrated developmental toxicity, reduced survival delayed growth and delayed hatching relative to controls. Survival rates were higher in BTMPS-exposed groups compared to xylazine; BTMPS also induced a dose-dependent delay in hatching, starting at 50 µM concentration with delays up to 24 hours. Conversely, xylazine had an insignificant effect on hatching at 72 hpf but demonstrated a higher toxicity with significantly higher coagulated embryos and reduced survival rate. Additionally, combinations of fentanyl, xylazine, and BTMPS produced greater developmental toxicity than individual compounds, including further delays in hatching. At the higher concentrations tested, all treatments induced physiological and/or morphological abnormalities.

Discussion: The findings of this study demonstrate that xylazine exhibits higher acute lethality compared to BTMPS but less than fentanyl. The synergistic effects of fentanyl and its emerging adulterants cause greater developmental toxicity than individual compounds and significantly disrupt developmental timelines in zebrafish. These findings highlight the need for expanded pharmacological studies to better understand toxicity and characterize the risks associated with fentanyl and its emerging adulterants.

Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

P-275

Opioid–Stimulant Co-Use in San Francisco: Trends in Overdose Mortality and Human Performance Toxicology

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Abstract

Introduction: In recent years, the opioid epidemic has evolved from predominantly opioid-only use to the widespread polysubstance use of opioids and stimulants. This combined opioid-stimulant use has emerged as a leading contributor to accidental drug overdose deaths (ADOD) nationwide. This study evaluated trends in combined opioid-stimulant use in ADOD in San Francisco from 2009-2025 and compared those trends with the toxicological findings in human performance (HP) cases.

Objectives: This study aimed to characterize the prevalence of opioids (fentanyl, heroin, and medicinal opioids) and stimulants (cocaine and methamphetamine) in ADOD investigations in San Francisco from 2009 to 2025. The observed trends in ADOD were further compared with the prevalence of opioid and stimulant detections in HP cases, including Impaired Drivers (DUID), Drug Facilitated Crime (DFC), and Public Intoxication (PI) cases.

Methods: A retrospective review was conducted of toxicological findings in ADOD and HP casework. Toxicological analysis included screening, confirmation, and quantitation of drugs by immunoassay, gas chromatography-mass spectrometry, and liquid chromatography-tandem mass spectrometry. The inclusion criteria for ADOD included an accidental manner of death, and opioids or stimulants included in the cause of death. The inclusion criteria for HP cases included positive detections for opioids or stimulants. Cases were categorized as opioid-only, stimulant-only, or combined opioid-stimulant use.

Results: Combined opioid-stimulant use increased throughout the study period. By 2025, the annual number of ADOD received represented a 300% increase from the number of ADOD received in 2009. Combined opioid-stimulant use accounted for 60% of ADOD in 2025 while opioid-only use and stimulant-only use each accounted for approximately 20%. That same year, combined opioid-stimulant use accounted for 32% of DUID cases, 36% of DFC cases, and 93% of PI cases. In 2025, no PI cases were opioid-only, compared with 12% of DUID and 7% of DFC. Stimulant-only use was observed in 56% of DUID cases, 57% of DFC cases, and 7% of PI cases.

Discussion: These findings reflect the progression of the fourth wave of the opioid epidemic in San Francisco. The increasing prevalence of combined opioid-stimulant use coincided with a substantial rise in ADOD. When compared to national data, the percentage of ADOD in San Francisco with combined opioid-stimulant use has been consistently greater than the national rate since 2021, indicating a disproportionately severe local impact.

The differences observed between opioid-only and stimulant-only use prevalence between ADOD and HP cases may reflect the increased lethality of opioids compared to stimulants in acute use. The window of detection also differs between case types, as blood is

predominately the only sample type submitted for DUID cases and urine is predominately the only sample submitted for PI cases. ADOD and DFC cases more commonly have both blood and urine submitted.

Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

P-276

Analytical Findings from a Consumer Case Involving a Triple-Chamber Cannabinoid Vape

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Abstract

Introduction: The unregulated cannabis industry has significantly expanded in recent years, from product availability to chemical composition, driven by regulatory gaps in the 2018 Agricultural Improvement Act. Product innovation is rapid to capture consumer market shares and labels are often incorrect in terms of concentrations and/or ingredient lists. The lack of clear, consistent oversight in the quality assurance of these products poses a risk to consumers for adverse effects. Vaping devices are typically equipped with a safety feature which automatically shuts the product off at 10 seconds in a single inhalation event. Vaping topography ranges from 3-10 seconds of inhalation, with an average of 4 seconds per puff.

Case History: An adolescent user experienced a seizure on school grounds. A BOUTIQ SWITCH V5 (3 in 1) “BAJA BERRY” (HYBRID) x “X-41” (HYBRID) x “DAYDREAM” (INDICA) cannabinoid device was found in their possession. The device was submitted to the Laboratory for Forensic Toxicology Research at Virginia Commonwealth University for characterization. It was a novel device with 3 pod chambers for 3 different vape formulations. The corresponding CoA found for the device was labeled to contain 76.38% delta-8-tetrahydrocannabinol (Δ8-THC), 7.33% delta-9-tetrahydrocannabiphorol (Δ9-THCP), 0.89% cannabinol (CBN), and terpenes. Prior cannabis consumption and medical history for the subject is unknown. The device had registered 9 “blinkers”. Vape “blinkers" refers to the number of times the device's battery was automatically turned off by the safety feature. This phenomena of counting how many times the device powers off has been popularized on social media as the “#blinkerchallenge”.

Objectives: The purpose of this experiment was to determine the chemical composition of the vape concentrate associated with an adolescent seizure case.

Methods: The case sample was evaluated for product characteristics including brand, flavor, and labeled ingredients/concentrations. The product was screened using a gas chromatography-mass spectrometry (GC-MS) method. Cannabinoid concentrations in the vapor formulations were determined using a previously validated liquid chromatography-tandem mass spectrometry (LC-MS/MS) method.

Results: Δ9-THC, Δ8-THC, CBN, cannabidiol (CBD), Δ8-iso-THC, tetrahydrocannanolic acid (THCA), d-limonene, linalool, fenchol, caryophyllene, humulene, and camphorene were identified in all three chambers. The average cannabinoid concentrations in the three chambers were: 61.1 ± 3.9% Δ9-THC, 1.3 ± 0.2% Δ8-THC, 1.7 ± 0.3% CBD, 0.34 ± 0.02% THCA, and <0.25% CBN.

Discussion: Excessive inhalation as recorded by the “blinker” count suggests potential high dose exposure. High doses of THC can cause anxiety, paranoia, confusion, hallucinations, nausea, hyperemesis, and loss of motor control. In rare cases seizures have been associated with children who consume high concentrations of THC. The “blinker” feature found on the BOUTIQ V5 device along with social challenges encourage high-dose aerosol inhalation. This practice warrants public health warnings to consumers and community stakeholders as to the dangers of high dose Δ9-THC consumption.

Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

Conflict of Interest

National Institute of Justice [15PNIJ-23-GG-014210COAP]. The opinions, findings and conclusions expressed are those of the author(s) and do not reflect those of the Department of Justice.

P-277

A Heuristic Evaluation of Point-of-Care Urine Tetrahydrocannabinol (THC) Test Kits

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Abstract

Introduction: Point-of-care (POC) drug testing kits are an accessible, rapid, and inexpensive way to test for drugs and/or their metabolites. POC urine drug test kits are advertised towards parents, schools, rehabilitation facilities, law enforcement, and clinical settings and come in various formats (e.g., test cups, dip cards/ test strips, cassette-style).

Delta 9-tetrahydrocannabinol (Δ9-THC) is the primary psychoactive compound in cannabis and is one of the most commonly used drugs in the United States. Due to its widespread use, Schedule 1 status, and impairing effects, Δ9-THC and/or its metabolites are frequently tested for criminal, substance use treatment, and workplace investigations

Presented is an overview of 43 POC THC urine drug test kits including: price, reported cut-off level, time for results, cross-reactivity, and other manufacturer provided information.

Objectives: To compare POC urine THC drug testing kits readily available in the United States and to provide an overview for consumers, practitioners, and those who are ultimately responsible for interpretation.

Methods: Data collection was performed via Google, Google Shopping and other online retail websites. Search terms included: “THC at home urine drug test,” “THC urine drug test,” “Marijuana urine test,” “Medical grade THC test kit,” and “Drug test kit for weed”. The following was recorded: product type, manufacturer, drug(s) targeted, price, marketing application, Food and Drug Administration (FDA) or Clinical Laboratory Improvement Amendments (CLIA) waived, cutoff concentrations, time to result(s), user reviews, and cross-reactivity details. Cross-reactivity data was obtained online and/or via communication with manufacturers.

Results: Forty-three products (test cups, dip cards/ test strips, or cassette-style) were identified from 36 different vendors/manufacturers; 18 were multi-drug panels. Prices ranged from $0.75 to $16.99 per test (mean = $9.71). Marketed applications included workplace testing, rehabilitation, at-home use, clinical settings, and law enforcement; 81.4% were FDA authorized and 88.37% were CLIA-waived. Reported cutoff concentrations were 50 ng/mL (n=29) and 15 ng/mL (n=5); one product offered multiple cutoffs by including multiple tests with different cutoffs and ten of the products did not report a cutoff. The time for results ranged from 3 - 5 minutes. Online reviews were available for 55.8% with ratings ranging from 2.3 - 5.0 (mean 4.1) out of 5 stars. Cross-reactivity was available online for 18 products. Cross-reactants included pantoprazole, omeprazole, efavirenz, naproxen, ibuprofen, cannabidiol, and cannabinol, as well as “hemp” products, vitamin B2 supplements, and Liverite.

Discussion: No consistent relationship was observed for price, marketed application, FDA-authorization, CLIA-waived status, user reviews, or cross-reactivity data availability. User reviews and cross-reactivity availability varied between products. Common cross-reactants included widely used medications and the full scope of cross-reactivity testing was not available for any product, limiting the interpretation/validity of results. This review of products will help clinicians, toxicologists, and others when attempting to understand POC results, limitations, and utility.

Disclosure

Yes, I, or a member of my immediate family, has a financial interest to disclose.

Conflict of Interest

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P-278

The Prevalence of Drug Poisoning and Overdose in Riyadh, Saudi Arabia

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Abstract

Introduction: Drug poisoning and overdose are growing public health challenges worldwide, contributing to preventable morbidity and mortality. Understanding their epidemiological patterns in specific settings is essential for developing effective prevention and intervention strategies. In Saudi Arabia, the use of all types of illicit drugs including alcohol is prohibited, and strict legal penalties are imposed on their trafficking or smuggling on a large scale. Despite this strict legal framework, recent years have witnessed a gradual shift towards recognizing drug abuse as a public health problem requiring prevention, treatment, and rehabilitation. This shift is reflected in the expansion of addiction treatment and rehabilitation services integrated within the health system.

Objectives: To assess the prevalence of drug poisoning and overdose cases in Riyadh, Saudi Arabia, and to identify the most commonly involved substances, as well as demographic risk factors including age and sex.

Methods: This retrospective observational study analysed hospital records, toxicology reports, and laboratory databases from medical institutions across Riyadh. The dataset included patients from diverse age groups and gender. Descriptive statistics were used to characterize demographic and clinical patterns, while regression analysis was performed to explore potential risk factors. Ethical approval was obtained from the Second Health Cluster, King Saud University, the Ministry of Health, and the University of Dundee.

Results: A total of 50,978 toxicology cases were analysed during the study period (2020–2024). Of these, 22,142 cases (43.4%) tested positive for at least one substance, while 28,836 cases (56.6%) were negative. The highest percentage of positive drug toxicity results was found among young adults (18-40 years old), with males being the most affected. Alcohol, THC and amphetamines being the most prevalent drugs detected overall. Samples included in this study originated from multiple referral pathways. Among those submitted through the forensic toxicology department, 17.8% tested positive for at least one substance, with alcohol, amphetamines, and THC representing the most commonly detected substances.

Discussion: These findings provide important insights into the scale and characteristics of drug poisoning and overdose in Riyadh. Identifying vulnerable populations can inform targeted prevention strategies and public health policies. Further research is recommended to explore underlying causes and evaluate intervention effectiveness, ultimately contributing to reducing drug-related harm in the region.

Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

P-279

Analysis of Confiscated Vaping Products from Public Schools Across the Commonwealth of Virginia During the 2025-26 Academic Year

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Abstract

Introduction: In the United States, the sale of tobacco-containing electronic cigarettes (vapes) has been restricted to consumers ≥21 years old since 2019. In May 2026, the United States Food and Drug Administration authorized the sale of vapes with flavors other than tobacco and menthol for the first time since 2020. There is no federal legislation specific to cannabinoid-based vaping products. National survey data from 2025 indicated that the percentages of U.S. adolescents vaping nicotine or cannabinoids daily were 3% and 1%, respectively, with most reporting using fruit-flavored vapes.

Objectives: To evaluate chemical compositions of vaping products used by Virginia's adolescent population during the 2025-26 academic year.

Methods: Vaping products confiscated from public school students were submitted to the Laboratory for Forensic Toxicology Research (LFTR) from all five health regions of Virginia. When possible, the student's grade level and how they acquired the device were submitted. Product type, advertised active ingredient, and brand and model of each device were catalogued in REDCap. Gas chromatography-mass spectrometry (GC-MS) was used to screen vape liquids. HeadSpace-gas chromatography-dual flame ionization detection (HS-GC-FID) was used to quantify volatile compounds. Liquid chromatography-tandem mass spectrometry (LC-MS/MS) was used to quantify cannabinoids. All methods have been previously published.

Results: Currently, 749 vaping products confiscated during the 2025-26 academic year have been received by LFTR. The most common device type was disposable (90.8%), followed by pod-replacement (8.3%) and refillable (0.8%). Of the disposable vapes (n=680), twenty-one were dual chamber (3.1%), and three were triple chamber (0.4%). The advertised active ingredient for the majority of vaping products was nicotine (82.8%), followed by cannabinoids (14.6%), unknown (2.0%), and zero nicotine (0.8%). Most vaping products were fruit-flavored (69.2%), while tobacco- or menthol-flavored products combined for 1.9% of submissions. Of submissions with grade information (n=539), most were confiscated from high schoolers (87.2%); the remainder were from middle schoolers (12.8%). The most reported methods of acquisition (n=116) were from a friend or peer (59.5%) or purchased by the student themself (17.2%). Of vapes that have been analyzed for volatiles so far (n=40), two contained ethanol at >1% by volume, including one dual chamber device containing ethanol >2.5% in both chambers. Untargeted analysis of nicotine vapes revealed high prevalence of nicotine benzoate salts and synthetic coolants. Cannabinoids identified via screening included, in order of prevalence, Δ9-tetrahydrocannabinol (Δ9-THC) Δ8-THC, Δ4(8)-iso-THC,

Δ8-tetrahydrocannabivarin (Δ8-THCV), Δ8-iso-THC, Δ9-THC acetate (Δ9-THC-O), 9(R)-, 9(S)-, and iso-hexahydrocannabinol (HHC), Δ6a,10a-THC, and Δ8-tetrahydrocannabutol (Δ8-THCB). Total phytocannabinoid concentrations in the vapes ranged from 49.4-84.0% by mass.

Discussion: These preliminary results show a continued prevalence of fruit-flavored e-liquids, synthetic coolants, nicotine salts, and cannabinoids - both natural and synthetic - in the vaping products confiscated from Virginia adolescents. The proportion of dual chamber devices submitted increased relative to previous confiscation years, and triple chamber devices are a new finding of this study. Only one vaping device submitted was an FDA authorized product. Taken together, these results highlight the ability of Virginia youth to access unregulated vaping products, and the myriad of risks associated.

Disclosure

Yes, I, or a member of my immediate family, has a financial interest to disclose.

Conflict of Interest

Grant/Research Support: This research was supported by the Virginia Foundation for Healthy Youth [8521496], National Institute of Justice [15PNIJ-23-GG-01421-COAP], National Institute on Drug Abuse [P30 DA033934], Virginia Commonwealth University's C. Kenneth and Dianne Wright Center for Clinical and Translational Research [UL1TR002649], & National Center for Advancing Translational Sciences [UL1TR002649]. The opinions, findings and conclusions expressed are only those of the author(s).

P-280

Trends in Multidrug Use Patterns: A 10-Year Comprehensive Analysis of Urine Drug Testing Reports

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Abstract

Introduction: Drug use patterns are dynamic and continuously evolving, driven by shifts in drug availability, socio-economic factors, and emerging psychoactive drugs.

Objectives: This study aims to evaluate the decade-long evolution of drug use patterns in Western Türkiye, with a specific focus on annual drug positivity trends, demographic shifts, and the emergence of complex multidrug use combinations.

Methods: A retrospective analysis was conducted on urine samples, comprising 45,244 urine toxicology reports collected between 2014 and 2024 at the Ege University Addiction Toxicology Laboratory. Urine samples originated from forensic (probation), clinical, and emergency department cases. Screening analyses were conducted using automated enzymatic immunoassays, and all positive findings were confirmed by chromatographic techniques. Drugs were categorized as continuously or periodically monitored according to evolving analytical panels. Data were systematically evaluated for annual positivity trends, demographic variations, and patterns of multidrug use. Multidrug use was defined as the presence of ≥2 drug classes. Statistical analyses, including chi-square testing and post-hoc evaluations, were conducted using R and SPSS, with significance set at $p<0.05$.

Results: The overall positivity rate was 29.4% (n=13,312). While testing volumes fluctuated—particularly declining during the COVID-19 period (2019–2021)—positivity rates remained relatively stable, peaking at 37.3% in 2020. Cannabis remained the most prevalent drug across the decade (up to 79.9% of drug test positive results were cannabis positive); however, a marked decline between 2019 and 2021 (to ~24–25%) coincided with a pronounced increase in amphetamine-type stimulants (ATS), particularly methamphetamine. This “cross-over” pattern strongly suggests a substitution effect in response to changing drug availability and market dynamics. A striking finding was the detection of pregabalin, introduced into the screening testing panel in 2023, with a high positivity rate of 49.3%, highlighting the rapid and underrecognized rise of prescription drug misuse. Concurrently, benzodiazepine positivity demonstrated a sustained increase over time, further supporting a shift toward pharmaceutical abuse. Multidrug use was identified in 18.1% of positive cases, with dual-drug use being predominant. The most frequent combination was ATS-cannabis (35.0%), followed by benzodiazepine-cannabis (10.8%) and ATS-benzodiazepine (8.3%), indicating a growing pattern of high-risk pharmacodynamic interactions. Demographically, the mean age was 27.2±10 years, with multidrug use significantly associated with younger individuals ($p=0.011$). Post-hoc analysis identified the 18–24 age group as a critical risk window, characterized by lower single-drug use and higher multidrug involvement. Gender analysis revealed that although males constituted the majority of cases (90.8%), females exhibited proportionally higher positivity rates for benzodiazepines and opiates, suggesting gender-specific drug use patterns.

Discussion: This 10-year longitudinal analysis demonstrates a clear transition from a cannabis-dominant landscape to a high-risk pattern characterized by the increasing prominence of amphetamine-type stimulants (ATS) and the growing misuse of prescription drugs, particularly pregabalin. The emergence of complex multidrug combinations—especially among young adults—underscores the need for adaptive toxicological screening strategies. Expanding routine panels to include emerging drugs and implementing age- and gender-specific public health and safety interventions are critical to effectively respond to the evolving drug use landscape.

Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

P-300

Direct Determination of Methamphetamine and Amphetamine in Urine by Thermal Desorption Counter-Flow Introduction Atmospheric Pressure Chemical Ionization Mass Spectrometry

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Abstract

Introduction: In the field of forensic medicine, the substances responsible for poisoning are diverse, and efficient preliminary screening prior to confirmatory testing is of great importance. We report the detection and quantitative estimation of methamphetamine (MA) and amphetamine (AP) in urine samples from forensic autopsy cases using a direct-introduction mass spectrometer. This approach may be particularly valuable because more traditional screening methods for amphetamines (i.e. immunoassay) have high cross-reactivity and lead to low confirmation rates.

Methods: Experiments were performed using a thermal desorption counter-flow introduction atmospheric pressure chemical ionization mass spectrometer (Hitachi DS-1000, Japan). Urine samples were diluted 10- or 100-fold with 10 mmol/L NaOH solution. A 20 µL aliquot of the diluted sample was spotted onto a cellulose non-woven wipe (Bemcot, Asahi KASEI, Japan) and loaded into the instrument. The compounds on the wipe were vaporized by heating on the block heater at 150°C, ionized by corona discharge, and analyzed in single MS and tandem MS modes.

Results: When urine samples spiked with MA and AP were analyzed, ions corresponding to the protonated molecules of each drug (*m/z* 150 for MA, *m/z* 136 for AP) were detected. Product ion spectra characteristic to the drugs were obtained in tandem MS mode, enabling drug identification. Calibration curves were constructed using the peak areas of the product ions *m/z* 150 > 119 for MA and *m/z* 136 > 119 for AP on the real-time traces. Excellent linearity (coefficients of determination (r^2) > 0.99) was observed for MA and AP within the range of 0.1–10 µg/mL in urine. Matrix effects were measured using negative control urine samples from 5 individuals. At an MA concentration of 0.5 µg/mL, the matrix effect of urine ranged from 80% to 121% (average 93%), while for AP, it ranged from 75% to 110% (average 95%). At the concentration of 5 µg/mL, the matrix effect for MA ranged from 83% to 126% (average 104%), while for AP, it ranged from 80% to 107% (average 94%). The method was applied to urine samples obtained from forensic autopsy specimens, and the correlation with quantitative data obtained using LC-MS/MS was evaluated. Of the 16 cases in which MA and AP were detected, urine samples from cases where the quantitative value of MA obtained by this method exceeded 10 µg/mL were diluted 100-fold with 10 mmol/L NaOH and remeasured in the same manner. The linear regression equations between the values obtained by this method and those by LC-MS/MS were y = 1.16 x (r^2 = 0.973) for MA and *y* = 1.02 x (r^2 = 0.967) for AP.

Discussion: Tandem mass spectrometry enabled the acquisition of characteristic product ion spectrum, allowing for the identification of the substances. It was possible to calculate the concentrations of MA and AP from the peak areas on the real-time traces. Although ion suppression and enhancement were observed due to individual differences in urine samples, the results obtained were sufficient for use as semi-quantitative values in rapid screening. We intend to continue measuring actual samples and comprehensively evaluate the practical applicability of the method.

Disclosure

Yes, I, or a member of my immediate family, has a financial interest to disclose.

Conflict of Interest

Grant/Research Support: This research was conducted with the equipment (DS-1000) provided free of charge by Hitachi High-Tech Solutions Corporation.

P-302

Evaluation of the Stability and Cross-Reactivity of Zopiclone and Eszopiclone Using Immunoassay Kits

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Abstract

Introduction: Zopiclone (ZOP) and its S-isomer, eszopiclone (EZOP), are hypnotics belonging to the cyclopyrrolone class, which are metabolized to zopiclone-N-oxide (ZOPNO) and N-desmethylzopiclone (NDZOP), respectively, and finally to 2-amino-5-chloropyridine (2A5C) after long-term storage, freezing, and conservation. This study aimed to examine the stability of ZOP and EZOP using a commercially available immunoassay urine screening kit for ZOP. ZOP and EZOP, as well as their metabolites and 2A5C, were analyzed in urine using liquid chromatography coupled with tandem mass spectrometry (LC–MS/MS).

Methods: Blank urine samples spiked with standards for ZOP, EZOP, and their metabolites, as well as clinical urine samples, were used to assess the cross-reactivity of the ZOP kit. The stability of clinical urine samples was tested under the following storage conditions: at 4°C, room temperature, and –30°C for up to 3 months. Their stability was also analyzed after three freeze (–30°C) and thaw (room temperature) cycles. The stability of the stored samples was initially assessed using the ZOP kit. Furthermore, the decomposition of each compound into 2A5C was confirmed using LC–MS/MS analysis. The concentrations of each compound were compared with those of the standard sample, which was stored at –30°C and analyzed simultaneously. This study has been approved by the Research Ethics Committee of Tokai University School of Medicine (approval number: 24R105).

Results: ZOP showed cross-reactivity at lower concentrations than EZOP. However, ZOPNO and NDZOP cross-reacted at half the concentration of ZOP. Moreover, we confirmed that after three months, ZOP, EZOP, and metabolites remained stable in all samples stored at –30°C. However, they appeared to be unstable at higher temperatures.

Discussion: The results indicate that urine samples stored for an extended period or altered postmortem may lead to false-negative results for ZOP, providing important information for interpreting results in cases involving ZOP. If the patient is in a febrile state after taking ZOP or EZOP, or if the patient is temporarily exposed to a hyperthermic environment after death, urinary 2A5C may be elevated. our findings show that this screening test is not always sufficient for accurately identifying these drugs. In forensic cases, especially cases involving the use of ZOP, a confirmatory test for 2A5C by instrumental analysis is necessary even in cases where the immunoassay is negative.

Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

P-303

Performance Evaluation of Antibody-Antigen Conjugate Pairs for Fentanyl and Norfentanyl Detection in Lateral Flow Assays

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Abstract

Introduction: Fentanyl remains a leading cause of overdose-related deaths, particularly in the United States. Sensitive detection is critical, as its life-threatening effects can be rapidly reversed by naloxone, the standard antidote for opioid overdose.

Fentanyl can be detected in urine, blood, and oral fluid samples of opioid users. Oral fluid is an attractive diagnostic matrix, as it reflects recent drug intake and contains the parent compound at detectable concentrations shortly after exposure, while urine remains relevant for longer detection windows due to the presence of fentanyl metabolites such as norfentanyl.

Among the various analytical methods available, lateral flow assays remain a key approach for detecting drugs of abuse use in oral fluid and urine. The sensitivity of these assays is highly dependent on the optimal pairing of antibody and antigen conjugate.

Objectives: The aim of the study was to characterize antibodies for the detection of fentanyl and norfentanyl, intended for use by diagnostic test developers in fentanyl IVD tests. To complement the monoclonal antibodies (mAb) in a competitive assay format, we also have developed fentanyl conjugates.

Methods: To evaluate the sensitivity of monoclonal antibody-conjugate pairs, bovine serum albumin (BSA) conjugates carrying fentanyl analogues were labelled with a fluorescent reporter and assessed in a competitive assay format against free fentanyl and norfentanyl. The most sensitive combinations were selected for further evaluation in a competitive lateral flow format.

In total, twenty antibody-conjugate pairs were screened in a competitive lateral flow assay using fentanyl as the target analyte. Selected candidates were subsequently tested in urine samples containing fentanyl and norfentanyl to assess performance in a relevant biological matrix.

Results: We report the performance of multiple high-affinity monoclonal antibodies demonstrating high sensitivity to fentanyl. The panel included both fentanyl-selective antibodies and antibodies with defined cross-reactivity to norfentanyl, enabling flexibility in screening strategies.

In competitive lateral flow assays, several antibody-conjugate pairs showed clear dose-dependent responses. The best-performing combination achieved sub-ng/mL sensitivity together with robust detection in urine. In addition, distinct antibody profiles were observed, including highly fentanyl-selective antibodies and others with broader cross-reactivity, supporting tunable assay design depending on the intended application.

Discussion: This study demonstrates the performance of monoclonal antibodies and antigen conjugates in a lateral flow format when used as matched pairs.

The results highlight that careful antibody selection enables tuning between fentanyl-specific detection and broader metabolite-responsive screening. Furthermore, pre-screening of antibody-conjugate combinations directly in the lateral flow format provides an efficient strategy for identifying optimal assay components. This approach supports the development of sensitive and adaptable diagnostic assays for fentanyl screening in real-world samples.

Disclosure

Yes, I, or a member of my immediate family, has a financial interest to disclose.

Conflict of Interest

Salary, From Medix Biochemica

P-304

Sensitive Quantification of Tetrodotoxin in Blood and Urine by LC-MS/MS

Zhang Xiaogang[1], Zhang Xiaojun[2], Yang Zong[1], Liu Bingjie[1], Adrian Taylor[3]

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Abstract

Introduction: Tetrodotoxin (TTX) is a highly potent neurotoxin most linked to pufferfish poisoning, though it has also been identified in other marine organisms and bacterial sources. By selectively inhibiting voltage-gated sodium channels, TTX disrupts nerve impulse transmission, resulting in rapid neurological symptoms such as perioral numbness, vomiting, respiratory distress, muscle paralysis, and in severe cases, death. Due to the toxin's extreme potency and rapid progression of toxicity, sensitive and reliable analytical methods are essential for confirmation of exposure in forensic investigations. Liquid chromatography–tandem mass spectrometry (LC–MS/MS) remains the preferred technique for trace-level determination of TTX in biological matrices.

Objectives: The aim of this study was to develop and validate a rapid, sensitive, and robust LC–MS/MS method for the determination of tetrodotoxin in human blood and urine, adapted and optimized from an established national forensic analytical standard. Main objective to optimize sample preparation to make it simple and convenient.

Methods: Blood and urine specimens (0.1 mL) were extracted using acidified methanol, followed by cleanup with mixed-mode solid-phase extraction cartridges. Chromatographic separation was achieved using hydrophilic interaction liquid chromatography under gradient conditions with aqueous acidic and organic mobile phases. Detection was performed using electrospray ionization in positive mode with multiple reaction monitoring (MRM). Matrix-matched calibration curves were prepared in both blood and urine. Method performance was assessed in terms of linearity, sensitivity, recovery, and intra-day precision.

Results: The method demonstrated excellent linearity over a concentration range of 0.05–200 ng/mL, with correlation coefficients exceeding 0.999. Mean recoveries ranged from 60.7–65.7% in blood and 88.2–93.5% in urine across all tested concentrations. Intra-day precision was satisfactory, with relative standard deviations below 5% in both matrices. The method provided sub-nanogram per milliliter sensitivity (LOQ 0.05 ng/mL) and a total chromatographic run time of 6 minutes per sample.

Discussion: This study describes a sensitive and efficient LC–MS/MS method for determining tetrodotoxin in blood and urine for forensic toxicology applications. The short run time and simplified sample preparation enable high-throughput analysis, while the method's sensitivity supports detection at forensically relevant concentrations. Although blood recoveries were lower than urine recoveries, TTX concentrations in routine poisoning cases are typically high, and approximately 60% extraction recovery is sufficient for qualitative screening. Blood TTX concentrations above 9 ng/mL are associated with a high risk of paralysis and respiratory arrest, while fatal cases have reported blood concentrations ranging from 12.8 to 119 ng/mL. Overall, the method provides a reliable tool for confirming tetrodotoxin exposure and supporting timely forensic investigations in suspected poisoning cases.

Disclosure

Yes, I, or a member of my immediate family, has a financial interest to disclose.

Conflict of Interest

Salary from company at which its products are being discussed

P-305

Rapid LC-MS/MS Determination of 2C-Series Phenethylamines and NBOMe Analogues

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Abstract

Introduction: Phenethylamine-based psychoactive substances represent an important class of new and emerging psychoactive drugs encountered in forensic toxicology. Structural modifications of the phenethylamine core have resulted in numerous potent hallucinogenic compounds, including the 2C series and their N-benzyl (NBOMe) derivatives. These substances exhibit high pharmacological potency, strong neurotoxicity, and significant abuse potential, and several have been implicated in severe intoxications and fatalities. Their structural similarity, low effective doses, and frequent misrepresentation as other drugs pose analytical challenges for forensic laboratories. Reliable, sensitive, and high-throughput analytical methods are therefore required to support monitoring, confirmation, and regulatory control of these compounds.

Objectives: The objective of this study was to develop and evaluate a rapid, sensitive LC–MS/MS method for the simultaneous determination of 13 phenethylamine drugs, including multiple 2C-series compounds and NBOMe derivatives, suitable for application in forensic toxicology and drug surveillance.

Methods: Chromatographic separation was achieved using reversed-phase liquid chromatography with gradient elution employing aqueous and organic mobile phases containing formic acid. Detection was performed using tandem mass spectrometry with electrospray ionization in positive mode and multiple reaction monitoring (MRM). Thirteen target analytes were included, encompassing 2C-H, 2C-D, 2C-E, 2C-C, 2C-P, 2C-B, 2C-I, 2C-T-2, 2C-T-7, and four corresponding NBOMe derivatives. Method performance was assessed through evaluation of chromatographic retention, limits of detection, linearity, recovery, and repeatability using matrix-matched samples. This method was not validated in accordance with ASB Standard 036 and there were no biological matrices used for this study.

Results: All 13 phenethylamine compounds were successfully separated and detected with good chromatographic retention and stable peak shapes. Sensitivity for all analytes reached the sub-picogram level, meeting analytical requirements for forensic applications. Matrix-matched calibration curves demonstrated excellent linearity across the tested concentration ranges, with correlation coefficients exceeding 0.99. Spike-recovery experiments at multiple concentration levels yielded recoveries between 88% and 113%. Method repeatability was evaluated using replicate injections, with relative standard deviations for peak areas below 4% across all compounds, indicating good precision and robustness.

Discussion: This study demonstrates a rapid and comprehensive LC–MS/MS method for the determination of a broad panel of phenethylamine drugs, including both classical 2C compounds and highly potent NBOMe analogues. The method addresses key analytical challenges associated with these substances, including structural similarity and low effective doses, while enabling high-throughput analysis suitable for routine forensic toxicology workflows. The approach provides a practical and reliable analytical tool for laboratories tasked with the detection, confirmation, and

monitoring of phenethylamine-based new psychoactive substances and supports ongoing drug surveillance and regulatory enforcement efforts.

Disclosure

Yes, I, or a member of my immediate family, has a financial interest to disclose.

Conflict of Interest

Salary from company at which its products are being discussed

P-306

Withdrawn

P-307

Development of a Highly Specific Competitive Immunoassay for the Quantification of Gabapentin in Human Blood Samples

Victoria Anderson, Ashleigh McLaughlin, Jonathan Mahoney, S. Peter FitzGerald

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Abstract

Introduction: Gabapentin, used medicinally as an anti-convulsant drug, is a structural analog of the inhibitory neurotransmitter gamma-aminobutyric acid (GABA). The drug is prescribed as adjunctive therapy in the treatment of epileptic seizures and as pain management of postherpetic neuralgia.

As of September 2025, recommendations for the toxicological investigation of Driving Under the Influence of Drugs (DUID) in the USA and Canada promoted gabapentin from a Tier II (optional) to a Tier I (recommended) compound due to increasing prevalence of abuse and the drug's potential to cause sedation, dizziness, and impairment to the user whilst not under clinical supervision.

Objectives: The aim of this study was to develop an Enzyme-Linked Immunosorbent Assay (ELISA) employing a new in-house, highly specific, monoclonal antibody for the detection and quantification of gabapentin in human blood samples.

Methods: A competitive ELISA was developed and evaluated which utilizes a highly specific monoclonal antibody developed for its specificity to gabapentin. The capture antibody was immobilized on a 96-well microtiter plate surface. Gabapentin (antigen), if present in the sample, competes with the horseradish peroxidase (HRP) labelled gabapentin (enzyme labelled antigen) for a limited number of antibody binding sites on the microtiter plate. Addition of an enzyme substrate initiates a colorimetric reaction, where enzyme activity and thus color intensity is inversely proportional to the level of drug present.

Absorbances are read at 450nm on a semi-automated plate reader, and a 6-point standard curve constructed to determine the concentration of gabapentin in the sample.

The limit of detection (LOD) in human blood was calculated from the mean concentration of 20 negative samples + 3 standard deviations. Accuracy was determined by spiking gabapentin into negative human blood samples at 3 concentration levels spanning the assay range, including at, 50% above and 50% below a commonly employed screening cut-off concentration (1000 ng/mL, 500 ng/mL and 1500 ng/mL). Three replicates of each level were assessed over 5 separate runs and the mean concentration, precision and percentage recovery calculated.

Results: The assay is standardized to gabapentin and showed < 1% cross-reactivity to the structurally similar gabapentinoid, pregabalin and other commonly associated cross reactants including: baclofen, 1,4-butanediol, valproic acid, GHB, (S)-N-methyl pregabalin, gamma-butyrolactone, methylhexanamine, γ-aminobutyric acid (GABA) and phenibut.

The assay was developed to achieve a cut-off value of 1,000 ng/mL, with an assay range of 16,000 ng/mL. The limit of detection (LOD) in human blood was 196.55 ng/mL. For each level assessed, percentage recovery was within 120.7% to 126.5%, while inter-assay precision was ≤11.6%. Intra-assay precision was determined to be ≤4.54%.

Discussion: This evaluation highlights the applicability of the ELISA, with the potential to perform as a highly specific and useful analytical tool in preliminary forensic and toxicological applications for the detection of low concentrations of gabapentin in human blood samples.

With the LOD determined below the cut-off concentration of 1,000 ng/mL and the assessment of precision samples showing optimal concordance whilst maintaining specificity to gabapentin, this ELISA could assist as a pivotal screening tool for forensic professionals in detecting and combating the increasing prevalence of gabapentin misuse and abuse within society.

Disclosure

Yes, I, or a member of my immediate family, has a financial interest to disclose.

Conflict of Interest

Salary

P-308

Evaluation of a New Multiplex Drugs of Abuse Immunoassay on the Evidence Rabta Analyser for Rapid Urine Testing

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Abstract

Introduction: Drugs of abuse screening is a critical component of forensic investigations to ensure the rapid detection of commonly abused substances such as opioids, amphetamines, cannabinoids, and cocaine. The constant rise in drug misuse has increased pressures on routine testing services, causing an increased demand for reliable, high-throughput screening methods that can be implemented to streamline testing and accelerate time to results. The development of new analysers, such as the Evidence RABTA (Random Access Biochip Technology Analyser), provides opportunities to advance drug testing through improved automation, continuous loading and random-access capabilities resulting in high throughput testing. Drug testing plays an important role in both clinical and forensic settings, with recent studies highlighting increasing demand for toxicological analysis.

Objectives: The objective of this study was to develop and evaluate an immunoassay screening kit for simultaneous detection of multiple drugs of abuse on the Evidence RABTA analyser. The study aimed to assess analyser and assay performance, including precision, recovery, carryover and included a method comparison using authentic human urine samples.

Methods: A multiplex biochip array for the following targets (cut off); Amphetamine (300ng/mL), Barbiturates (200ng/mL), Benzoylecgonine (300ng/mL), Methamphetamine (200ng/mL), Opiates (300ng/mL), Oxazepam (200ng/mL), THC (25ng/mL), Tramadol (200ng/mL) and Creatinine (as an adulterant marker), was applied to the fully automated Evidence RABTA analyser. Intra-assay precision was evaluated by analyzing two samples - one with a concentration below the assay cut-off and one above the cut-off (n=15 for each sample). Inter-assay precision was assessed using the same samples, analysed in triplicate over 5 days (n=15) to determine assay reproducibility. Recovery was determined by analysing low, medium, and high concentration samples, each ran in triplicate. Concentrations were compared to target values and percentage recovery calculated. Analyser carryover was assessed by analysing 3 replicates of a high concentration sample, followed by 3 replicates of a low concentration sample. A qualitative method comparison of 20 authentic urine samples was performed for 7 of the assays on the array. Samples were analysed on the Evidence RABTA analyser using the developed immunoassay biochip array and compared against an alternative method (Analyser Architect). Percentage agreement between the methods was assessed to evaluate the analytical performance of each assay.

Results: Precision was determined to be ≤8% and ≤8.5% for all assays in intra- and inter-assay determinations, respectively. No evidence of carryover was observed during the process of the evaluation. Recovery was between 81% and 101% across all of the analytes on the panel. For the authentic sample method comparison, percentage agreement was 100% for the 7 assays included in this study. This included a range of authentic positive and negative samples; methamphetamine and tramadol were excluded from the method comparison as the 20 samples used in the study had not been analysed for these targets.

Discussion: The Evidence RABTA analyser, along with the developed immunoassay kit, demonstrated a high level of performance across key validation criteria such as precision and recovery. No evidence of sample carryover was observed during the evaluation process, which has been eliminated through the design of the analyser, reducing the potential for misclassification of sample results. Furthermore, all assays assessed showed 100% agreement with the reference method.

Disclosure

Yes, I, or a member of my immediate family, has a financial interest to disclose.

Conflict of Interest

Salary

P-309

Easy Strategy for the Addition of New NPS Compounds: Differentiation of Methylfentanyl Isomers and Isobaric Compounds

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Abstract

Introduction: Efficient NPS surveillance demands a flexible framework that integrates new compounds quickly with minimal validation and no changes to extraction or chromatography. Since 2019, a streamlined LC-MS/MS screening method (Garneau et al., 2021 *Forensic Science International*, 318:110595) has allowed for frequent, rapid updates.

Objectives: During the eighth update of the NPS screening method, 15 substances were added. However, specificity issues arose for ortho-methylfentanyl due to isobaric and isomeric compounds; the implemented solutions are described here.

Methods: Blank urine and blood samples, spiked with 69 NPS compounds, underwent protein precipitation with acetonitrile/acetone. Separation was performed on a Zorbax C18 column using a pH 3.0 ammonium formate/acetonitrile gradient. A SCIEX 6500 MS (positive mode) identified analytes via retention time (±0.05 min) and ion ratios (±30%) across two mass transitions.

Results: The presence in the panel, and thus the standard solution, of the isomer cis-3-methylfentanyl, and isobaric compounds butyryl fentanyl and isobutyryl fentanyl, created interference with ortho-methylfentanyl. While full chromatographic resolution was achieved with butyryl fentanyl (RT: 7.27 min, ion ratio[1]: 5.1), ortho-methylfentanyl (RT: 7,00 min, ion ratio[1]: 26.3) was not fully resolved from cis-3-methylfentanyl (RT: 7.07, ion ratio[1]: 9126.7), which itself was not fully resolved from isobutyryl fentanyl (RT: 7.13 min, ion ratio[1]: 5.1). Unresolved compounds contributed to each other's transitions, compromising identification.

During compound optimization for this method, all detectable fragments for each substance are evaluated and recorded in a table. This summary table could be used here to assess whether an alternative set of transitions for the different compounds could offer improved selectivity for each isomer, without compromising sensitivity.

Among the 11 transitions tested for cis-3-methylfentanyl and ortho-methylfentanyl, only the fragment at m/z 150 Da was selective for cis-3-methylfentanyl. In contrast, for ortho-methylfentanyl, the transitions at m/z 188 Da and 146 Da demonstrated sufficient selectivity.

Discussion: In this case, full selectivity for cis-3-methylfentanyl could not be achieved in the presence of ortho-methylfentanyl. Evaluation of cis-3-methylfentanyl prevalence in casework between 2019 and 2025 showed that this compound had never been detected in any sample. Consequently, this compound was removed from the method and the reference solution. If an interfering peak is observed at ortho-methylfentanyl's transitions, but fails to meet ion ratio acceptance thresholds, the presence of cis-3-mehylfentanyl can be suspected and confirmed

using an alternative method. The ion ratio of this interfering peak can be used to differentiate between cis-3-methylfentanyl and isobutyryl fentanyl interferences.

This evaluation was also extended to trans-3-methylfentanyl and para-methylfentanyl, and demonstrated acceptable selectivity for ortho-methylfentanyl.

Ion ratio is thus a useful tool to differentiate isobaric compounds, in addition to chromatographic resolution. To that effect, it is useful to record all possible transitions for future reference when carrying out compound optimization.

[1] Ion ratio at *ortho*-methylfentanyl selected transition

Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

P-310

Field-Based Quantitative Analysis of Cannabis Samples via Portable Near-InfraRed Spectroscopy

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Abstract

Introduction: The rapid identification and quantification of cannabinoids in cannabis samples are essential for forensic and regulatory purposes. While traditional chromatographic methods such as LC-MS and GC-MS offer high accuracy, they are often time-consuming and require extensive laboratory preparation. Portable Near-InfraRed (NIR) spectroscopy presents a promising, non-destructive alternative for on-site analysis, facilitating more efficient forensic workflows.

Objectives: This study aims to develop and validate a localized multivariate calibration model for the quantitative analysis of cannabinoids in cannabis samples using portable NIR spectroscopy. A key focus is to evaluate the performance of these models against commercial cloud-based alternatives and to assess the impact of incorporating samples spiked with synthetic and semi-synthetic cannabinoids on model robustness.

Methods: Over 300 cannabis samples were analyzed using two portable MicroNIR On-Site-W spectrometers, with each sample subjected to at least six replicate measurements to ensure reproducibility. A dedicated subset of approximately 70 samples seized by Czech authorities and containing synthetic or semi-synthetic cannabinoids was analyzed via High-Resolution Mass Spectrometry (HRMS) to generate precise reference data. Spectral data were preprocessed and utilized to develop a localized multivariate calibration model in The Unscrambler X, which was compared against a commercial NIRLab cloud-based model. Reference concentrations for major cannabinoids (CBD, CBDA, Δ9-THC, and Δ9-THCA) were obtained through validated LC-MS and GC-MS analyses to serve as the ground truth for model training and validation.

Results: The localized NIR models quantified CBD and THC with acceptable predictive performance, as assessed by RMSEP and R^2, covering a broad concentration range from 0.1 to 20.1 for CBD and from 0.0 to 37.3 for THC, while several synthetic and semi-synthetic cannabinoids were identified qualitatively. The developed localized NIR models demonstrated high accuracy in the quantification of cannabinoids; however, their robustness was lower than that of the generalized commercial NIRLab model when measurements were performed under conditions different from those used for model calibration, such as indirect measurements through glass or plastic or analysis of samples deviating substantially from the calibration set. Nevertheless, the localized models offered the advantage of fully local processing without dependence on cloud connectivity, which is particularly beneficial in field settings, and the inclusion of samples containing synthetic and semi-synthetic cannabinoids in the calibration set improved the model's ability to distinguish diverse cannabis varieties and cannabinoid profiles.

Discussion: These results underscore the viability of portable NIR spectroscopy as a rapid, accurate, and cost-effective tool for the forensic quantification of cannabis. By utilizing site-specific multivariate calibration models, forensic laboratories and law enforcement agencies can improve throughput and enable rapid, on-scene decision making without the need for destructive sample preparation. Future research will focus on expanding the adulterant and synthetic cannabinoid library and further optimizing models to account for natural matrix variability.

Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

Conflict of Interest

This study was supported by the Ministry of Interior of the Czech Republic (Project No. VK01010137).

P-311

Assessment of Authentic Postmortem Vitreous Humor Biochemistry by Nova Prime Plus

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Abstract

Introduction: Vitreous humor (VH) electrolyte analysis assists forensic pathologists in identifying potential metabolic contributors to death. Because VH is anatomically isolated and less affected by postmortem change, its biochemical stability supports interpretation of antemortem metabolic state. With limited laboratory bench space, the Nova Prime Plus offers a compact alternative to larger analyzers for VH analysis.

Objectives: This study aims to assess and validate the Nova Prime Plus for VH analysis by comparing performance to reference laboratory methods and evaluating suitability for forensic casework.

Methods: Instrument validation included external accuracy, reproducibility, and analytical measurement range (AMR) assessment by Nova Biomedical, followed by internal validation performed by the Forensic Laboratory Division of the San Francisco Office of the Chief Medical Examiner. Internal studies followed ANSI/ASB Standard Practices for Method Validation in Forensic Toxicology and evaluated applicability, dilution integrity, reproducibility, and precision. Proficiency test samples, quality controls (QCs), and authentic VH samples were analyzed. Dilution integrity was tested using saline, deionized water, and HEPES/LiOH buffer. Reproducibility and precision were assessed through repeated analysis of authentic VH samples and QCs.

Results: Across four proficiency test sets, the Nova Prime Plus met CAP acceptance criteria for sodium, potassium, chloride, glucose, creatinine, and urea nitrogen (UN). Biases occurred primarily when analyte concentrations were near the instrument's limit of quantitation (LOQ) or exceeded the upper limit of quantitation (ULOQ), particularly for glucose and creatinine.

Comparison of authentic VH case samples to reference laboratory results showed consistent agreement (<20% difference) for sodium, potassium, chloride, and most glucose values. The instrument detected low-level glucose (<15 mg/dL) in cases where the reference laboratory reported "None Detected." In contrast, creatinine and UN showed substantial variability (–42% to +337%) with no consistent trend.

Analysis of 20 additional centrifuged VH samples demonstrated similar patterns for sodium, potassium and chloride remaining within 20% agreement, while creatinine and UN varied across their measurement ranges regardless of concentration. During this period, the reference laboratory's updated reporting of glucose as "<10 mg/dL" improved comparability between instruments.

Dilution studies showed that deionized water lowered ionic strength and prevented analysis, while saline removed sodium and chloride, making results unsuitable. A HEPES/LiOH buffer successfully restored ionic strength and resolved "No Air When Required" errors, though diluted case samples showed more variability than diluted QCs.

Discussion: Validation results indicate that the Nova Prime Plus is suited for VH electrolyte analysis, demonstrating strong performance for sodium, potassium, chloride, and glucose. Variability in creatinine and UN appears driven by differences in analytical differences (ISE vs. spectrophotometry), potential VH non-homogeneity, and timing between analyses, suggesting careful interpretation of these analytes, especially near LOQ/ULOQ boundaries.

Dilution studies confirmed that traditional diluents were incompatible with the analyzer's ionic strength requirements. Although HEPES/LiOH buffer effectively enabled analysis, diluted case samples exhibited variability, reflecting the complex composition of VH. Dilution should therefore be used only when needed to resolve analyzer errors or results exceeding ULOQ.

The Nova Prime Plus provides a compact, bench-friendly instrument with reliable performance for key VH electrolytes, supporting its use in forensic metabolic assessment while acknowledging limitations for creatinine and UN interpretation.

Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

P-312

Determination of Pregabalin in Biological Samples (Blood, Urine) by LC-MS/MS

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Abstract

Introduction: Pregabalin is a widely used calcium channel modulator for neuropathic pain, partial-onset epilepsy, and generalized anxiety disorder, and is a key controlled drug in therapeutic drug monitoring (TDM) and forensic toxicology. Due to low concentrations and complex matrix interference in biological samples, existing methods [1] exhibit significant discrepancies in sensitivity (LOD ranging from 19.00 μg/mL to 4.9 ng/mL), selectivity, and applicability across matrices, making them insufficient for rapid clinical TDM and trace pregabalin identification in drug-related cases. Establishing an efficient and accurate quantitative detection method for pregabalin in biological samples is therefore of great practical importance.

Objectives: This study aims to develop a sensitive, accurate, and rapid QTRAP LC-MS/MS method for precise quantification of pregabalin in blood and urine, providing scientific and reliable technical support for clinical TDM for individualized dosing, forensic toxicological trace identification, and related pharmacological and toxicological research.

Methods: Blank biological samples were collected from healthy volunteers, and blood and urine test samples were obtained after simulated drug administration. Sample pretreatment was performed using Waters Oasis MCX solid-phase extraction cartridges. Briefly, 0.2 mL of sample was pretreated with 0.1 mL of 0.1% formic acid in methanol; the supernatant after high-speed centrifugation was loaded onto the cartridge, which was then washed sequentially with 1 mL of water and 1 mL of methanol and pressurized to dryness. The analyte was eluted with 0.5 mL of 5% ammonia-methanol, evaporated to dryness, and reconstituted with 200 μL of 0.1% formic acid aqueous solution. Chromatographic separation was achieved on a C18 column with gradient elution using 0.1% formic acid in acetonitrile and water. QTRAP LC-MS/MS was operated in positive electrospray ionization mode with MRM-IDA-EPI and secondary spectral library retrieval. Deuterated pregabalin was used as the internal standard.

Results: Method validation showed good linearity for pregabalin in both blood and urine over 1–200 ng/mL, with $r^2 > 0.999$. The LOD was 0.5 ng/mL and LOQ was 1.0 ng/mL. Extraction recovery exceeded 95.9% with RSD < 15.0% (n=6). Matrix effect was above 85.0%, and intra-day and inter-day precision RSDs were both within 15%. The method effectively removed endogenous matrix interference in blood and urine, fully meeting detection requirements for pregabalin in biological samples.

Discussion: The established QTRAP LC-MS/MS method features a simple workflow, short pretreatment time, high sensitivity, excellent quantitative accuracy, good precision, and minimal matrix interference, enabling efficient separation and trace quantification of pregabalin in blood and urine. Compared with traditional spectroscopic and HPLC methods, it effectively addresses large detection deviations for low-concentration pregabalin and high false-positive rates in complex matrices. The method fully satisfies trace detection requirements in routine clinical

TDM, forensic toxicological identification of drug-related cases, and related pharmacological and toxicological research, demonstrating high practical value for clinical individualized administration guidance and judicial adjudication of drug-related cases.

Reference

[1] Malviya, N. J., Alqarni, M., Alshehri, A. A., Obaydo, R. H., & Varu, H. L. (2025). Evolution of Pregabalin Analytical Methods: A Comprehensive Assessment of Performance, Accessibility, and Future Directions. Critical Reviews in Analytical Chemistry, 1–21.

Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

Conflict of Interest

Research Support

P-313

Withdrawn

Withdrawn

P-314

The Use of a Novel Quadrupole Time-of-Flight Instrument for the Quantitative and Sensitive Analysis of Drugs of Abuse in Hair Samples

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Abstract

Introduction: The detection and quantification of drugs in hair present significant analytical challenges, particularly with respect to sensitivity and matrix effects. Tandem quadrupole mass spectrometers are typically employed for targeted analyses due to their high sensitivity and the specificity afforded by multiple reaction monitoring (MRM). However, high resolution mass spectrometry (HRMS) instruments offer additional advantages, including enhanced selectivity for compounds with similar m/z values and improved discrimination against background interferences.

Objectives: The objective of this study was to expand upon our previous work (1), in which a panel of drugs in hair was analyzed using a tandem quadrupole mass spectrometer. In the present study, a novel quadrupole time of flight (QTof) mass spectrometer with a multiple reflecting flight-tube was evaluated for the accurate identification and quantification of a panel of commonly encountered drugs of abuse. The goal was to demonstrate that the cutoff criteria recommended by the Society of Hair Testing (SoHT) could be met using this instrument and methodology.

Methods: Decontaminated hair samples were pulverized, weighed into vials (20 mg), and incubated in a proprietary solvent mixture for 2 hours. Calibrators and quality control samples were created by adding known working standards to pulverized hair. Internal standards were added to all samples prior to incubation. Samples were subsequently extracted using mixed mode cation exchange solid phase extraction plates (Waters Oasis™ MCX Plates), as previously described (1) with minor modifications. LC–MS/MS analysis was performed using a Waters ACQUITY™ Premier System coupled to a Waters Xevo™ MRT Mass Spectrometer operated in Tof MRM mode, in which precursor ions are fragmented and analyzed by the time of flight analyzer, leading to highly specific quantitation. For this proof of concept method, 13 drugs and metabolites including natural and synthetic opioids, amine stimulants, benzodiazepines and hypnotics were analyzed. These are listed in Table 1.

Table 1. Analytes included in this analysis

Opioids	Amine Stimulants	Benzodiazepines	Other
Morphine	MDA	Nordiazepam	Ketamine
Codeine	Methanphetamine	Diazepam	Norketamine
6-acetyl morphine			
Norfentanyl			
Norbuprenorphine			
Fentanyl			
EDDP			

Results: The modified extraction procedure enabled a substantial reduction in final sample volume without compromising extraction recovery or overall method performance, resulting in a corresponding increase in analytical sensitivity. For example, extraction recoveries were equivalent for many compounds (within 10%) and substantially greater for several, such as the amine stimulants. Calibration curves encompassed the cutoff concentrations recommended by the Society of Hair Testing and the European Workplace Drug Testing Society. Quantitative results demonstrated acceptable accuracy and precision for all analytes. At the recommended SoHT cutoffs, accuracy values ranged from 85-120% with all but 12/13 within 15%. Precision values ranged from 4-27% with 11/13 under 20%.

Discussion: A sensitive proof of concept method was developed for the quantitative analysis of drugs of abuse in hair using Tof MRM on a novel high resolution time of flight mass spectrometer. The method demonstrated accuracy and precision at the SoHT cutoff criteria, highlighting the applicability of this novel high resolution time of flight instrument for targeted forensic hair analysis.

1-Danaceau et al. Extraction and Analysis of a Definitive Drug Panel in Hair Samples by UHPLC-MS/MS for Forensic Toxicology Waters Application note, 2025 720008769en.

Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

P-316

Assessment of Acquisition Strategies for Qualitative Drug Screening Using the SCIEX ZenoTOF 7600

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Abstract

Introduction: Accurate identification of drugs in biological samples is a core challenge in forensic toxicology, particularly as the diversity of compounds encountered in casework continues to expand. The emergence of novel psychoactive substances, designer drugs, and widespread prescription drug use has increased the need for screening approaches that are both comprehensive and adaptable. Liquid chromatography high-resolution mass spectrometry (LC–HRMS) supports broad-scope screening by providing accurate-mass measurements and compound specific MS/MS information across a wide range of analytes.

Objectives: The aim of this study was to evaluate a qualitative LC–HRMS screening workflow on a ZenoTOF 7600 using multiple acquisition strategies including data-dependent acquisition (DDA), and data-independent acquisition (DIA) in both positive and negative ionization modes. The data analysis focuses on automated processing methods and minimal manual intervention.

Methods: Biological matrix samples were prepared using a simple protein precipitation approach and used for qualitative screening analyses. A reversed-phase chromatographic method was developed to enable rapid screening, with a total analysis time of less than 15 minutes. Acquisition on the ZenoTOF 7600 included DDA and DIA workflows. Data processing incorporated exact mass screening, isotope pattern evaluation, MS/MS spectral matching, and automated qualitative filtering using commercially available and in-house spectral libraries. Internal standard monitoring and flagging tools were applied to support data quality assessment with minimal manual review.

Results: Using the qualitative screening criteria applied in this workflow, broad compound coverage was observed across quality control materials and representative samples. DIA strategies provided improved performance for select compound classes compared to data-dependent acquisition, particularly where more complete MS/MS information supported confident identification. Enhanced MS/MS spectral quality with Zeno trap activation allowed for improved library matching for lower-abundance analytes. Compounds detected spanned multiple drug classes including opioids and cannabinoids, as well as additional drugs of abuse and pharmaceuticals. Overall, the results highlight the flexibility of the LC–HRMS workflow and demonstrate how acquisition strategy and MS/MS quality influence qualitative screening outcomes in forensic toxicology applications.

Discussion: These results demonstrate that acquisition strategy has a measurable impact on screening outcomes, particularly for compound classes where MS/MS completeness and sensitivity influence library matching and identification confidence. The observations highlight practical considerations for LC-HRMS screening implementation, including acquisition strategy selection, application of scoring and filtering criteria, and integration of automated data processing to support transparent, criteria-based interpretation. The flexibility of the workflow allows for these acquisition and data processing strategies to be adapted based on laboratory needs, target scope, and emerging drug trends.

Disclosure

Yes, I, or a member of my immediate family, has a financial interest to disclose.

Conflict of Interest

Salary

P-317

A Robust and Automated LC–MS/MS Workflow for Quantitative Urine Drug Testing Across a Broad Dynamic Range on a Triple Quadrupole Mass Spectrometer

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Abstract

Introduction: Robust and high-throughput LC-MS/MS workflows for urine drug testing are essential for clinical and forensic laboratories to ensure accurate detection across a wide dynamic range while minimizing carryover and manual intervention.

Objectives: In this study, we evaluated the analytical performance of an LC–MS/MS method developed on a triple quadrupole mass spectrometer for the quantitation of pain management drugs and drugs of abuse using hydrolyzed urine matrices representative of real-world clinical samples.

Methods: Calibration standards were prepared in hydrolyzed synthetic urine fortified with a panel of pain management drugs and drugs of abuse. A calibration curve (n = 5) spanning from 25 ng/mL to 1000 ng/mL to assess linear dynamic range and quantitative performance. Quality control samples were independently prepared at 25 ng/mL and 100 ng/mL in hydrolyzed urine to evaluate method accuracy and reproducibility. A total of 79 drugs of abuse and metabolites were monitored using 181 MRM transitions, with at least two transitions per analyte. Multiple reaction monitoring (MRM) transitions were optimized for all analytes using OptiFlow ion source parameters to maximize sensitivity and robustness. To mitigate analytical carryover and reduce the need for manual review, the innovative SCIEX OS software dynamic decision rules were implemented to automatically trigger blank injections following detection of carryover events.

Results: Baseline chromatographic separation was achieved for challenging isomer compounds, such as morphine/hydromorphone and codeine/hydrocodone), demonstrating that the short 3 min gradient did not comprise chromatographic resolution. Method performance was assessed using a series of hydrolyzed urine-spiked calibrators as well as two QC samples. All compounds were successfully detected and quantified within the validated linear range (25-1000 ng/mL), although the lower limit of quantification (LLOQ) varied by compound. These results demonstrate the method's suitability for complex, multi-analyte urine testing workflows. No carryover was observed, which was defined as 1/3 of the lowest calibrator.

Discussion: Overall, this approach demonstrated a sensitive, automated, and clinically relevant urine drug testing workflow that combined optimized MRM acquisition with software-driven decision rules. The accelerated MRM (aeMRM) technology allowed for faster MRM acquisition times, ultimately enabling a 3 min runtime. The results highlight the potential for improved laboratory efficiency, reduced carryover risk and reliable quantitation across a broad concentration range in routine clinical practice.

Disclosure

Yes, I, or a member of my immediate family, has a financial interest to disclose.

Conflict of Interest

Salary from SCIEX

P-318

Practical Implementation of a Single-Platform Software Workflow for Forensic Toxicology Screening to Confirmation

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Abstract

Introduction: Forensic toxicology laboratories routinely employ a two-step workflow consisting of broad drug screening followed by targeted confirmatory analysis. These workflows often rely on multiple analytical platforms, software packages, and data processing approaches, which require additional training, increase operational complexity, and create potential inefficiencies in data review and reporting. To address these challenges, this study evaluates a single software platform that supports both screening and confirmatory toxicological analyses across LC-MS and GC-MS techniques, providing a streamlined workflow from initial detection through final confirmation within a single data analysis environment.

Objectives: To evaluate whether a single software platform enables screening-to-confirmation workflows combining spectral library searching and targeted compound lists for LC-MS and GC-MS, which will reduce the analyst time to do data processing.

Methods: Screening was performed using a 5-minute LC-MS/QTOF method. Confirmatory analysis was primarily conducted using LC-MS triple quadrupole (TQ) instrumentation with forensic toxicology databases. Method performance was demonstrated by spiking 50 analytes and 23 internal standards into biological matrices, including synthetic urine and human blood. These compounds include, but are not limited to, NPS drugs. GC-MS was additionally evaluated as a complementary confirmatory technique to provide laboratories with flexibility when GC-MS instrumentation and existing workflows are available. The GC-MS Scan/SIM method was performed using an RXi-5MS column (30 m), successfully separating over 40 analytes, including internal standards. Chromatographic resolution was acceptable if the measurement from the shared valley to the baseline was less than 10 percent of the measurement from the target analyte peak to the baseline. Two libraries, including the Toxicological Cayman library, were used for compound identification based on full-scan mass spectral matching with 80% similarity index threshold. Confirmation of target compounds was performed using a compound table that included retention time, target and reference ions, and ion-ratio criteria. This combined method was then applied to urine samples to evaluate quantitation performance. Calibration curve ranges were compound depended. Library searching, targeted compound list screening and quantitation were all performed within a single software platform (Insight Explore) to enable positive identification in the samples.

Results: Fifty drugs of abuse, including isobars such as morphine and hydromorphone as well as codeine and hydrocodone, were separated on a Shim-pack Scepter PFPP-120 column (1.9 µm, 2.1x50 mm) utilizing a gradient elution from 55-100% with 10 mM ammonium formate and 0.1% formic acid as additives in water (mobile phase A) and methanol (mobile phase B). The compounds were analyzed by positive and negative mode electrospray ionization (ESI) on both an LC-MS QTOF and TQ LC-MS. For the QTOF analysis, compounds were identified by library-matching against forensic toxicology databases. For LC-MS confirmation, the Shimadzu forensic toxicology database was used. Instrument detection limits were determined in solvent for the rapid screening method on the QTOF and the forensic toxicology method package methods on the TQ.

Discussion: This approach demonstrates that consolidating screening, library matching, targeted compound confirmation, and quantitation into a single platform can streamline toxicology workflows, reduce operational complexity, and improve efficiency while maintaining reliable identification and separation of structurally similar compounds in biological matrices.

Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

P-319

The Analysis of 12 Cannabinoids in Whole Blood by LC-MS/MS

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Abstract

Introduction: In addition to the psychoactive cannabinoid THC, there is a growing interest in researching the potential medicinal benefits of the plant's other major cannabinoids which are non-psychoactive. Cannabinoids are very hydrophobic compounds which elute late in reversed phase chromatography. Since phospholipids also elute in this region, matrix effects and ion suppression can be a concern for the analysis of these compounds.

Objectives: This research presents an LC-MS/MS method for the analysis of 12 cannabinoids and 2 metabolites from whole blood. The objective is to present a simple sample preparation technique utilizing a phospholipid removal cartridge to facilitate the removal of phospholipids, reduce matrix effects, and achieve a good recovery while avoiding the added inconvenience of a dry down step.

Methods: Whole blood samples were prepared by protein precipitation with and without phospholipid removal. Protein precipitation samples were prepared by slowly adding 1000uL of chilled acetonitrile with internal standard to 250ul of whole blood while vortexing, followed by 10 minutes of centrifugation at 13,500 rpm. The supernatant from these protein precipitation samples were then injected and analyzed. The phospholipid removal samples followed the same initial protocol, when the supernatant was removed, 15µL of concentrated formic acid was added and loaded into a "Phree" phospholipid removal cartridge and the eluate was collected and injected. Analyte recovery was tested at 100 ng/mL as the ratio of the mean peak area of pre-extraction spiked whole blood samples divided by the mean peak area of post-extraction spiked whole blood samples (N=3 for both sets).

The separation was performed on an Agilent 1260 HPLC system using a Phenomenex Luna Omega Polar C18 column (50 x 2.1mm, 3 µm, 00B-4760-AN). Mobile phase A (MPA) and mobile phase B (MPB) were 10 mM ammonium formate with formic acid in water and formic acid in methanol, respectively. The total HPLC runtime was 10 minutes. Mass Spectral analysis was performed on the SCIEX QTRAP 4500 System.

Results: The Luna Omega Polar C18 Column allowed for the analysis of 12 cannabinoids and 2 metabolites in 10 minutes, while resolving 4 groups of isobaric cannabinoid species. The Polar C18 column chemistry provided sufficient retention of these compounds to allow for direct injection of the eluate, avoiding the need for a dry down step. Recovery was comparable between protein precipitation and Phree extracted samples with a 1-13% difference in recovery between techniques for all analytes except delta-9-THC, which had a 20% difference. A qualitative comparison of phospholipid content using several known MRM transitions shows protein precipitation leaves behind a large amount of phospholipids around 5 minutes. The phospholipid removal cartridge removes the majority of these phospholipids. Protein precipitation samples showed a 127-197% increase in the area ratios for these compounds compared to neat standards, while Phree-extracted samples matched the neat standards within ± 10% with the exception being delta-9-THC which was around 70%.

Discussion: The work here demonstrates an LC-MS/MS method for the analysis of 12 cannabinoids and 2 metabolites from whole blood. Adding phospholipid removal after protein precipitation significantly reduced matrix effects which is especially important when stable isotope-labeled analogs are not available for each compound.

Disclosure

Yes, I, or a member of my immediate family, has a financial interest to disclose.

Conflict of Interest

I am an employee of Phenomenex

P-320

A Case of Suspected Fentanyl Laced with Bromethalin Rodenticide

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Abstract

Introduction: A recent death investigation suggested a 26-year-old female used bromethalin-laced fentanyl prior to being located with agonal breathing. Bromethalin is a neurotoxin found in the rodenticide Tomcat Mouse Killer. Bromethalin rapidly metabolizes via N-demethylation to the more potent neurotoxin, desmethylbromethalin (DMB). Bromethalin converts to DMB in the mass spectrometer, so DMB analysis was explored. A method for its detection was developed as it is not included in routine toxicology screening.

Objectives: Bromethalin exposure was suspected. The objective of the study was to develop LC-MS/MS and LC-Orbitrap HRMS analytical methods enabling extraction and detection of DMB in postmortem whole blood.

Methods: DMB powder (LGC) (1000 mg/mL; CH_3OH) was used to prepare an infusion solution (200 ng/mL, 50%A:50%B), a neat standard (1000 ng/mL, 20%B) and spiked whole blood (1.6 to 100 ng/mL). Warfarin-d5 (Cayman Chemical) was used as internal standard (IS) (200 ng/mL; CH_3CN). Whole blood (200 uL) containing 50 ul of IS was precipitated using 2x100 uL aliquots of CH_3CN. Supernatant (200 uL) was removed, dried under N_2 and reconstituted in 100 uL of 80%A:20%CH_3CN. Extracts (20 uL) were injected on both LC-MS/MS and LC-HRMS. DMB detection (negative mode) on an Agilent 1260 LC-Sciex 3200 MS/MS was optimized by direct infusion and an Allure PFP Propyl 5.0µm, 50 x 2.1mm column was used. A Thermo Vanquish LC-QExactive Plus HRMS used Full MS/ddMS2 with targeted monoisotopic masses of 559.77095 m/z (DMB) and 312.12897 (warfarin-d5). Analytical separation was accomplished using a Raptor Biphenyl 2.7mm, 150 x2.1mm column. Aqueous(A) and organic(B, CH_3CN) mobile phases were equivalent on both instruments, each containing 0.2% formic acid and 2 mM ammonium formate, with gradients starting at 20%B. LC gradients increased to 95%B over 9 mins (LC-MS/MS), and to 98%B over 9.4 min (LC-HRMS), using flow rates of 0.5 mL/min.

Results: Screening methods using both LC-MS/MS and LC-HRMS were developed. LC-MS/MS MRM transitions $[M-H]^-$ were m/z 312.2 > 255.0 and 161.0 for warfarin-d5 and 561.8 > 278.2 and 253.7 for DMB. Retention times were 4.51 min (IS) and 7.19 min (DMB) on the LC-MS/MS and 4.50 (IS) and 6.30 min (DMB) on the LC-HRMS. The extractability of DMB in whole blood enabled satisfactory detection to 1.6 ng/mL on both instruments meeting all detection requirements. Analysis of postmortem cardiac blood (grey top tube) for the decedent was negative for DMB.

Discussion: Human bromethalin fatalities are rare. A lethal case involved ingestion of 17 mg, 0.33 mg/kg, of bromethalin. Animal studies of bromethalin suggest rapid GI absorption, peaks at 4h in serum and has a $t_{1/2}$ of 5.6 d. Suitable detection by LC-MS in whole blood was accomplished using pure and spiked standards at low and high concentrations to allow analysis of an authentic case sample. The case specimen was negative for DMB. This process identifies that due

diligence is required when investigating unusual toxicology cases and the necessity to define the capabilities of the instrument and extraction method to detect the analyte of interest at relevant concentrations.

Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

P-321

Withdrawn

P-322

Understanding the Metabolism of Ethylbromazolam Using Human Liver Microsomes

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Abstract

Introduction: Ethylbromazolam is a novel psychoactive substance (NPS) structurally similar to the known illicit benzodiazepine Bromazolam. This ethyl analogue emerged on the illicit drug market in late 2024 / early 2025, with reports of detection in multiple countries throughout 2025 and 2026. Much has been reported about Bromazolam in terms of its pharmacology and toxicology, however, there is limited data available for this new ethyl analogue. Ethylbromazolam has not been approved for medical use and its presence in counterfeit pharmaceuticals poses a health risk.

Objectives: The goal of this study is to identify probable phase I metabolites of the novel designer benzodiazepine Ethylbromazolam through incubation with human liver microsomes (HLMs), followed by liquid chromatography and high-resolution mass spectrometry analysis.

Methods: Ethylbromazolam was incubated with pooled HLMs. Metabolites were analyzed using UPLC coupled to the electrospray ionization (ESI) source of a Orbitrap Exploris mass spectrometer scanning in selected ion monitoring (SIM) mode with ddMS2 for targeted analysis of specific *m/z* of anticipated metabolites. The potential accurate *m/z* values of interest and the corresponding MS/MS spectra were extracted based on anticipated methods of metabolism for other related benzodiazepines. Authentic reference standards of plausible metabolites were synthesized and used to support findings.

Results: This study revealed that the metabolism of Ethylbromazolam resembles that of Bromazolam and similar benzodiazepines of this sub-class. By comparison to authentic reference standards, we can report that hydroxylation occurs primarily at the alpha position as well as the 4-position on the scaffold yielding isobaric structures sharing a common *m/z* of 383.05.

Discussion: Designer benzodiazepines are becoming more prevalent in illicit drug marketplaces and have begun to show up in toxicological samples. Identification of their primary metabolites will aid toxicologists in interpreting casework and give them the tools they need to develop appropriate methods for detection.

Disclosure

Yes, I, or a member of my immediate family, has a financial interest to disclose.

Conflict of Interest

We work for a private company. We will be presenting original research and not promoting products, however.

P-323

Analysis of 7-Hydroxymitragynine and Mitragynine in Urine Workplace Drug Testing Specimens at CRL

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Abstract

Introduction: *Mitragyna speciosa*, or kratom, is a plant native to Southeast Asia that has been used for centuries for its dose-dependent effects, counteracting fatigue and improving work productivity as a psychostimulant, and relieving pain and substituting for opium as a sedative analgesic. Kratom became widely available in the United States in the early 2000s, reached mainstream status around 2015, and was noted as a public health concern by the US Drug Enforcement Administration in 2016. The two main psychoactive components in kratom are mitragynine and 7-hydroxymitragynine (7-OH), both of which affect μ-, δ-, and κ-opioid receptors. Due to increased affinity at the μ-opioid receptor, 7-OH is estimated more than 20-times more potent than mitragynine, and at least 13-times more potent than morphine. In certain kratom strains, mitragynine can comprise up to 66% of total alkaloids; 7-OH makes up less than 2% of naturally occurring plant alkaloids, but is also a product of mitragynine metabolism. As a result of dosing with the kratom plant, concentrations of 7-OH in urine are typically 24-27% of corresponding mitragynine concentrations.

Objectives: Determine the prevalence of 7-OH and mitragynine in non-regulated workplace drug testing samples and show the effect of 7-OH on confirmation rates and positive-reporting samples when categorized by reason for test.

Methods: In August 2025, 1012 random urine specimens were de-identified and analyzed for 7-OH and mitragynine. This initial study yielded 10 positives for both 7-OH and mitragynine, and one positive for only 7-OH. Given the 0.99% positivity rate for mitragynine and the 1.09% positivity rate for 7-OH, 7-OH was added to the existing mitragynine analytical panel and made available for client request in November 2025.

Results: Evaluation of data from November 2025 through February 2026 revealed a higher positivity rate, with more than 1.5% reporting positive for one or both analytes. The overall confirmation rate for the 7-OH/mitragynine panel was 95.3%, with 68.8% of positive samples reporting positive for both analytes. Of reported positives, 19.7% were positive for only 7-OH, while 11.5% were single mitragynine positives. Notably, 65.6% of positive samples had 7-OH concentrations higher than mitragynine concentrations, likely indicating the use of 7-OH as opposed to kratom. The most prevalent reasons for testing among reported positive samples were Random tests, at 65.6%, and tests due to Reasonable Cause, at 9.8%; of the positive samples listed for Reasonable Cause, all had higher concentrations of 7-OH than mitragynine.

Discussion: With a more than 1% positivity rate for randomly-selected, previously unscreened specimens, and an even greater rate of confirmation among specifically requested sample testing, the presence of 7-OH and mitragynine in urine workplace drug testing samples alludes to a serious public safety threat that is not being addressed. In 2026, the legal status of kratom and its derivatives continues to evolve, with bans and/or Schedule 1 status in 8 states, but with unrestricted availability in at least 20 other states. Regardless of legal status, more employer

drug testing programs should consider the inclusion of 7-OH and mitragynine due to substantial intoxication effects, addiction potential, and community and personal welfare.

Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

P-324

First Detection of Four 2-Oxo Arylcyclohexylamine Analogues in Korea: Forensic Identification and Drug Trend Analysis

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Abstract

Introduction: Arylcyclohexylamine analogues such as phencyclidine (PCP) and ketamine are known to exert dissociative and hallucinogenic effects primarily through N-methyl-D-aspartate (NMDA) receptor antagonism. Continuous subtle structural modifications of the arylcyclohexylamine core, driven by efforts to circumvent legal controls, have led to the emergence of novel psychoactive substances (NPS) whose toxicological risks remain poorly characterized. Novel arylcyclohexylamine analogues have been continuously emerging worldwide, and the pace of their appearance has been accelerating in Korea as well.

Objectives: This study aimed to characterize the structures of four newly detected 2-oxo arylcyclohexylamine analogues and to investigate current NPS trends in Korea based on statistics of the National Forensic Service (NFS).

Methods: A small portion of each unknown substance, unmatched in existing spectral libraries, was dissolved in methanol and analyzed by combined analytical techniques. The possible structures were first predicted using molecular m/z values and fragmentation patterns from gas chromatography–mass spectrometry (GC–MS). Definitive structural confirmation was subsequently achieved by high-performance liquid chromatography–quadrupole time-of-flight tandem mass spectrometry (HPLC–QTOF–MS/MS) and nuclear magnetic resonance (NMR) spectroscopy.

Results: Following the first detection of 3-MeO-2-oxo-PCP in Korea in August 2025, three additional analogues — 2-oxo-PCP, 2-oxo-PCPr, and 2-fluoro-2-oxo-PCiP — were subsequently identified. 2-oxo-PCP bears a piperidine ring and a β-keto moiety on the cyclohexyl portion; 3-MeO-2-oxo-PCP incorporates an additional methoxy substituent at the 3-position of the phenyl ring; and 2-oxo-PCPr and 2-fluoro-2-oxo-PCiP carry propyl and isopropyl substituents on the amine group, respectively, with the latter further distinguished by a fluorine substituent at the 2-position of the phenyl ring. With the exception of 2-oxo-PCP, which was first recovered in e-cigarette liquid, the remaining three analogues were seized as white powders initially suspected to be ketamine. According to statistics of the NFS, a total of 97 cases involving the four newly identified analogues were submitted between August 2025 and the end of April 2026. While most seized items consisted of powder samples or associated drug paraphernalia, those related to 2-oxo-PCP included liquid samples, their containers, and e-cigarette devices. Various other controlled substances, such as ketamine, etomidate, and synthetic cannabinoids, were found to be co-circulating with these analogues. In terms of age distribution, abuse was most prevalent among individuals in their 20s and 30s, with a higher proportion of male cases than female cases.

Discussion: The pace of structural diversification among NPS in Korea is accelerating. Although direct potency data are still lacking, the structural features of these analogues place them within the ketamine-like subfamily. Related 2-oxo analogues such as 2-oxo-PCE have been linked to a cluster of severe acute intoxications. How these structural modifications affect potency, however, remains

uncertain and requires direct pharmacological evaluation. To enable interdiction at the initial stage of domestic distribution, it is essential to build a rapid structural identification system and to maintain continuous forensic surveillance through timely information sharing among relevant agencies.

Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

P-325

Metabolic Evaluation of the Synthetic Opioid U-47700 Using In Vitro and In Vivo Approaches for Forensic Toxicology Applications

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Abstract

Introduction: Legal highs present a great threat to health, especially in groups of people experimenting with psychoactive substances. The lack of available knowledge on the biotransformation of these substances necessitates symptomatic treatment in the event of intoxication, which, unfortunately, may be ineffective. Opioids, including heroin analogues, such as U-47700, constitute a special group of designer drugs. These compounds are associated with severe intoxications, including fatal cases, and remain a significant challenge for modern forensic toxicology.

Objectives: In this study, a multi-directional approach to trace the biotransformation of U-47700 in living organisms was used. The objective was to better understand metabolic pathways of this compound and to improve the interpretation of toxicological findings in clinical and forensic contexts.

Methods: For this purpose, the in silico assessment (ADMET Predictor) was used first and then followed by an in vitro study using human liver microsomes and the S9 fraction. The biotransformation was then followed in an animal model (Wistar rats). Tissues such as blood, brain and liver were collected for analysis. The study was performed using liquid chromatography with tandem mass spectrometry (LC-MS/MS). Additional experimental steps included time-dependent incubation and dose-dependent *in vivo* exposure to assess metabolic dynamics.

Results: The obtained results were compared to those obtained from the analysis of autopsy materials (cases analysed in the Toxicology Laboratory of the Department of Forensic Medicine, Jagiellonian University Medical College in Krakow). The results indicated that the major metabolic pathways of U-47700 involve N-demethylation and hydroxylation, leading to the formation of U-47700 metabolites such as N-desmethyl-U-47700 and N,N-didesmethyl-U-47700. The observed metabolic profiles were consistent across in silico, in vitro, and *in vivo* models, and showed good agreement with authentic forensic case data.

Discussion: The applied multidirectional approach provides valuable insight into the metabolism of synthetic opioids and demonstrates the usefulness of combining different experimental models. The identification of U-47700 metabolites is essential for improving toxicological screening and interpretation of intoxication cases. These findings highlight the importance of integrated research strategies in forensic toxicology and support their application in the evaluation of emerging new psychoactive substances.

Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

P-326

Forensic Detection of Nitazenes in Ontario: A Retrospective Analysis of Fatal Toxicity and Impaired Driving Cases (2019-2025)

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Abstract

Introduction: The opioid crisis remains a major public health concern in North America, with highly potent synthetic opioids contributing substantially to morbidity and mortality. Benzimidazole-derived opioids, commonly referred to as nitazenes, were first synthesized in the 1950s but were never approved for clinical use due to their extreme potency and high abuse potential.

Objectives: This study aimed to characterize the prevalence of nitazenes detected in forensic toxicological specimens in Ontario, Canada, and to describe patterns of co-detection with other substances in both death investigation and suspected drug-impaired driving cases.

Methods: A retrospective analysis was conducted of all toxicological specimens analyzed at the Centre of Forensic Sciences (CFS) in Ontario between October 2019 and December 2025. Nitazenes were detected by Quadrupole Time of Flight (QTOF) mass spectrometry with specific nitazenes being added to the library throughout the study period (Isotonitazene being the first in October 2019, with periodic additions through to protodesnitazene in October 2025). Cases were categorized as Office of the Chief Coroner (OCC) death investigations or suspected drug-impaired driving cases. Nitazene prevalence was assessed on both a monthly and annual basis, and co-detected substances were analyzed to identify common drug combinations.

Results: There were 276 distinct cases positive for nitazenes within the study period, representing a low but meaningful proportion of overall toxicology submissions. The 276 cases included 193 OCC death investigations and 83 impaired driving cases. Eleven nitazene analogues (or metabolites) were detected: metonitazene, isotonitazene, protonitazene, etodesnitazene, N-pyrrolidino protonitazene, N-desethyl isotonitazene, N-pyrrolidino etonitazene, isonitazene, N-desethyl protonitazene, 5-aminoisotonitazene, and N-pyrrolidino metonitazene. Overall metonitazene (115 detections) and isotonitazene (72 detections) were the most frequently identified. Nitazene detections increased precipitously from 2020 to 2023, peaking in late 2021 to early 2022, followed by stabilization then decrease through 2025. Detection levels in 2025 were ~70% of that seen during the peak period in 2021-2022 and appeared to be stabilizing by the end of the study period. Co-detection with other substances was common across both case types. Fentanyl was frequently co-detected, along with other central nervous system depressants such as benzodiazepines (notably flualprazolam), as well as stimulants including methamphetamine and cocaine metabolites.

Discussion: Nitazenes represent a highly potent class of synthetic opioids in forensic toxicology casework and contribute to the ongoing opioid crisis. Their frequent co-detection with fentanyl and other central nervous system depressants underscores the risks associated with an increasingly complex and unpredictable unregulated drug market, with heightened potential for adverse effects including impairment and fatality. Interpretive challenges arise primarily from limited toxicological

data—including information related to potency, pharmacokinetics, metabolism, and the absence of routine quantitative results—rather than from co-detection alone. While analytical capability for drug quantitation is well established, gaps remain in the timely development and routine implementation of methods across laboratories. Continued surveillance is essential to monitor synthetic opioids circulating in the population to ensure accurate interpretation, curate public health responses, and monitor the evolving landscape of synthetic opioid use.

Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

P-327

Cardiotoxicity of Chloromethcathinones (CMC) in Postmortem Studies.

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Abstract

Introduction: Synthetic cathinones, which include chlorometcathinone isomers, account for over 50% of analytically confirmed cases of novel psychoactive substance (NPS) use detected at the Department of Forensic Medicine, Jagiellonian University Medical College. It is noteworthy that in recent years, chloromethcathinone isomers (3- or 4-CMC) have been the most frequently detected NPS in studies conducted by the local toxicology laboratory. As inhibitors of norepinephrine and dopamine reuptake, CMCs affect the circulatory system and may cause acute or chronic cardiac damage as observed in postmortem microscopic examination.

Objectives: The objective of this study was to assess the potential for CMC-induced cardiotoxicity through histopathological evaluation of heart specimens in cases associated with the ingestion of these substances.

Methods: The study group consisted of 20 individuals aged 16–44 years in whom the presence of one of the CMC isomers was confirmed analytically and who exhibited one to three features of cardiac damage on histopathological examination. Toxicological analyses of blood, urine, and liver samples were performed using a previously published and validated LC-ESI-MS/MS method capable of differentiating 3-CMC and 4-CMC and detecting their dihydro metabolites [1]. Histopathological examinations were performed on formalin-fixed heart sections stained with standard eosin and hematoxylin and additionally subjected to CD3 immunohistochemical staining.

Results: In the cases described, features of acute cardiac injury were identified. In two cases, mixed-cell infiltrates with a predominance of neutrophils in contact with altered fibers were observed; in one case, focal neutrophilic infiltration was found; in two cases, single-fiber necrosis was present; and in six cases, necrosis with contracture nodules was identified. Changes indicative of a chronic nature were also demonstrated. In one case, a focus of fresh replacement fibrosis was observed; in three cases, a focus of replacement fibrosis was found; in six cases, stromal cell proliferation/stromal edema was present; and in one case, hypertrophic cardiomyopathy was identified. In all cases, the presence of CMC and/or its metabolite was confirmed. In four cases, the 4-CMC isomer was detected along with its metabolite, dihydro-4-CMC; in eight cases, only the 4-CMC metabolite was detected; in three cases, the 3-CMC isomer was detected along with its metabolite, dihydro-3-CMC; and in five cases, only the 3-CMC metabolite was detected.

Discussion: Preliminary studies have shown a trend supporting the cardiotoxic potential of chloromethcathinone isomers. However, the presence of CMCs is not invariably associated with histopathological evidence of cardiac injury, and cases with confirmed exposure but no detectable cardiac abnormalities have been observed in forensic practice. Only cases demonstrating histopathological features of cardiac injury were included in this study. Furthermore, the study population consisted of relatively young individuals whose age and available medical records did not suggest pre-existing cardiovascular disease. Nevertheless, the limited sample size and retrospective design preclude establishing a direct causal relationship between CMC exposure and the observed

cardiac lesions. Further studies involving larger cohorts are needed to better characterize the cardiotoxic potential of chloromethcathinone isomers.

[1] Romańczuk et.al The Stability of Synthetic Cathinones and the Study of Potential Intake Biomarkers in the Biological Material from a Case of 3-CMC Poisoning, *Journal of Analytical Toxicology*, Vol. 47, Issue 5, 2023, 470–480, https://doi.org/10.1093/jat/bkad010

Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

P-328

Withdrawn

Withdrawn

P-329

Synthesis and Analysis of Metabolites of Hexahydrocannabinol (HHC) and Its Analogs

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Abstract

Introduction: Hexahydrocannabinol (HHC), a well-known semi-synthetic cannabinoid, has been widely abused globally since its appearance on the drug market in 2022. Subsequently, various HHC analogs emerged in which a *n*-alkyl chains ranging from C3 to C8 were attached to the 3-position of HHC, and these have also been abused. In Japan, generic scheduling was introduced in 2023 to regulate analogs of Δ^9-tetrahydrocannabinol (Δ^9-THC), Δ^8-THC, and HHC. For HHC analogs, this means that HHCV, HHCB, HHC, HHCH, HHCP, and HHCjd (HHC-octyl), are controlled, as well as their *O*-acetate derivatives.

Objectives: In forensic science, it is currently unclear which metabolite is the optimal urinary marker to prove the ingestion of HHC and its analogs. In this study, we synthesized (8*R*, 9*R*)- and (8*S*, 9*S*)-8-OH-metabolites of HHCV, HHCB, HHC, HHCH, HHCP, HHCjd (HHC-Octyl), to use them as reference standards for certifying the intake of these semi-synthetic cannabinoids, and investigated whether these metabolites could be differentiated using gas chromatography/mass spectrometry (GC/MS).

Methods: Δ^8-THC-OTf, in which a triflate group is introduced at the 3-position of Δ8-THC, was converted to (8*R*,9*R*)- and (8*S*,9*S*)-8-OH-Δ^8-THC-OTf via a hydroboration-oxidation reaction. Next, (8*R*,9*R*)- and (8*S*,9*S*)-8-OH-Δ^8-THC-OTf were reacted with the corresponding boronic acid via the Suzuki-Miyaura coupling reaction to introduce a *n*-alkyl chain from C3 to C8 at the 3-position. The synthetic metabolites obtained in this way and their trimethylsilyl (TMS) derivatives were analyzed using GC/MS, and their fragmentation patterns were examined.

Results: All synthesized (8R,9R)- and (8S,9S)-8-OH-metabolites exhibited distinct retention times on the GC column, both as free forms and TMS derivatives. The mass spectra of the TMS derivatives of all (8R,9R)-8-OH-metabolites showed multiple fragment ions, including the molecular ion ($M^{+\cdot}$), *m/z* $[M-133]^+$, and *m/z* $[M-105]^+$. In contrast, the TMS derivatives of all (8S,9S)-8-OH-metabolites showed *m/z* $[M-105]^+$ as the base peak ion alongside the $M^{+\cdot}$. Based on these mass spectral differences, all (8*R*,9*R*)- and (8*S*,9*S*)-8-OH-metabolites could be distinguished.

Discussion: The fragmentation patterns of the (8*R*,9*R*)- and (8*S*,9*S*)-8-OH-metabolites were different. This is due to the difference in stereochemistry at the 8-, 9-positions. In our previous work, we synthesized all four 8-OH-isomers, (8*R*,9*R*)-, (8*S*,9*S*)-, (8*S*,9*R*)-, and (8*R*,9*S*)-8-OH-HHC, and revealed that their mass spectra could be classified into two fragmentation patterns based on the difference in stereochemistry at the 8-position, regardless of the stereochemistry at the 9-position. Therefore, the observed patterns suggest that the fragmentation depends on the stereochemistry of the hydroxyl group at the 8-position. These synthesized compounds may be used as reference standards of metabolic markers to certify the intake of semi-synthetic cannabinoids, such as HHCV, HHCB, HHC, HHCH, HHCP, and HHCjd (HHC-Octyl). The GC/MS

data from this study are expected to provide useful information for metabolic studies of HHC and its analogs.

Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

P-330

Miniaturized Salting-Out-Assisted Liquid-Liquid Extraction Coupled to LC-MS/MS for the Quantification of Nitazene Analogs in Plasma

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Abstract

Introduction: Nitazene opioids are a rapidly expanding class of highly potent synthetic opioids associated with severe intoxications and fatal poisonings. Their structural diversity, typically low concentrations in biological specimens, and limited toxicological interpretation data create significant challenges for clinical and forensic toxicology laboratories. Sensitive and validated quantitative methods are therefore essential to support case investigation, toxicovigilance, and toxicological interpretation.

Objectives: This study aimed to develop and validate a miniaturized salting-out-assisted liquid-liquid extraction (SALLE) method coupled to liquid chromatography-tandem mass spectrometry (LC-MS/MS) for the quantification of 38 compounds: nitazene analogs and related metabolites in human plasma, and to evaluate its applicability in an authentic intoxication case.

Methods: Sample preparation was performed using a miniaturized SALLE procedure. A 100 µL aliquot of human plasma was mixed with 10 µL of internal standard solution, followed by the addition of 400 µL of acetonitrile and 100 mg of partitioning salts composed of sodium acetate and magnesium sulfate (NaAc:$MgSO_4$, 1:4, w/w). Samples were vortex-mixed for 5 min at 2500 rpm and centrifuged for 5 min at 14,000 rpm. The supernatant was collected and evaporated to dryness under a nitrogen stream at 40 °C, and the residue was reconstituted in 100 µL of methanol. A 5 µL aliquot was injected into a UHPLC system coupled to an LCMS-8060 triple quadrupole mass spectrometer (Shimadzu), operating in positive electrospray ionization mode. Chromatographic separation was achieved on a C18 column (50 mm × 3.0 mm, 2.6 µm) using gradient elution with water and methanol, both containing 0.1% formic acid and 2 mM ammonium formate. Detection was performed in multiple reaction monitoring mode, with two transitions monitored per analyte. The method was validated according to forensic toxicology guidelines, including selectivity, carryover, linearity, limits of detection and quantification, bias, precision, matrix effects, recovery, and stability.

Results: The method provided sensitive and reliable quantification of nitazene analogs in plasma over a concentration range of 0.05-20 ng/mL, with coefficients of determination greater than 0.99. The limit of detection was 0.025 ng/mL and the lower limit of quantification was 0.05 ng/mL. Bias ranged from -2.6% to 15.7%, while within-run and between-run precision ranged from 3.4% to 12.7% and from 4.4% to 14.9%, respectively. No relevant endogenous or exogenous interferences and no carryover were observed. Matrix effects and recovery varied among analytes. Although fentanyl was used as a single surrogate internal standard, quantification was supported by matrix-matched calibration and analyte-specific validation of bias and precision. Under these conditions, the observed matrix effects and recovery did not compromise the predefined acceptance criteria. The validated method was successfully applied to an authentic severe intoxication case, in which

N-pyrrolidino protonitazene was quantified in plasma at 1.4 ng/mL. The clinical presentation included marked central nervous system depression, miosis, apnea, severe oxygen desaturation, and reversal after repeated naloxone administration.

Discussion: The validated miniaturized SALLE-LC-MS/MS method provides a sensitive, low-volume, and practical approach for targeted quantification of emerging nitazene opioids in plasma. Its successful application to a severe intoxication case demonstrates clinical and forensic relevance and supports the inclusion of nitazenes in analytical toxicology workflows, particularly in regions where these compounds are newly emerging.

Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

P-331

Structure Characterization of Newly Emerging Cannabinoids: THC-P and HHC-P

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Abstract

Introduction: The forensic landscape of new psychoactive substances has been reshaped by the rapid market emergence of semi-synthetic cannabinoids such as tetrahydrocannabiphorol (THC-P) and hexahydrocannabiphorol (HHC-P) and their stereoisomers. Their substituents differ from those of the canonical Δ^9-THC structure, conferring an approximately 30-fold higher affinity for the CB1 receptor. Knowledge of analytical reference data — such as detailed 3D structure, absolute configuration and spectral profiles of these molecules — therefore remains highly desirable for international forensic databases.

Objectives: The aim of this work is to provide the first comprehensive vibrational (FT-IR, Raman) and chiroptical (ECD, VCD) reference spectra for (9R)-HHC-P, (9S)-HHC-P, Δ^8-THC-P and Δ^9-THC-P, further supported by quantum-chemical density functional theory (DFT) modelling, in order to assign the absolute configuration and detailed 3D structure of these molecules in solution.

Methods: Reference standards (purity > 98 %) were measured in methanol and as neat solids using FT-IR (transmission and ATR), dispersive Raman (785 nm excitation), UV/ECD and FT-VCD spectrometers. A systematic conformational search was performed by rotating the dihedral angles of the side chains in 120° steps, followed by full geometry optimisation and harmonic frequency / rotational-strength calculations at the B3LYP/6-311+G(2d,p) level using the polarizable continuum solvation model. Boltzmann-weighted spectra were assembled from all conformers populated at ≥ 4 % at room temperature and compared with the experimental data through normal-mode assignment.

Results: The DFT calculations identified 5 stable conformers populated at ≥ 4 % for (9*R*)-HHC-P, 13 for (9S)-HHC-P, 10 for Δ^8-THC-P and 9 for Δ^9-THC-P; the diastereoisomers differed primarily in the orientation of the heptyl chain and in the arrangement of methyl substituents. The Boltzmann-weighted simulated IR, Raman, UV, ECD and VCD spectra reproduced the experimental band positions, intensities and signs with an excellent fit, allowing complete vibrational mode assignment in the 1800–800 cm^{-1} (IR/Raman) and 1600–1200 cm^{-1} (VCD) regions. Marker bands discriminating Δ^8- from Δ^9-isomers and (9R)- from (9S)-epimers were identified; the sign of the VCD couplet at 1450 cm^{-1}, together with the ECD Cotton effect at 270–290 nm, enabled unambiguous assignment of the absolute configuration.

Discussion: To the best of our knowledge, this work represents the first complete UV, ECD, VCD, IR and Raman reference dataset for THC-P and HHC-P stereoisomers, validated using state-of-the-art DFT calculations. The combined vibrational–chiroptical workflow is non-destructive, reagent-free and applicable to small (< 20 mg) seized samples, offering forensic toxicology laboratories an independent tool for the confirmation of these cannabinoids.

The reference spectra and DFT-optimised geometries will be made available to the forensic community upon request.

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Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

P-332

Cytotoxic, Genotoxic, and Oxidative Stress–Mediated Toxicity of Synthetic Cathinones: Evidence from Experimental Models

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Abstract

Introduction: Synthetic cathinones (β-keto phenethylamine derivatives) are the second largest group of new psychoactive substances (NPS), structurally related to cathinone and designed to mimic classical stimulants such as amphetamine. Existing data remain fragmented across disparate experimental models and have not been systematically integrated to characterize organ-specific toxicity mechanisms or structure-activity relationships.

Objectives: This review systematically evaluates the cytotoxic, genotoxic, and oxidative stress–mediated effects of synthetic cathinones in experimental models, with emphasis on hepatic, neuronal, and cardiac toxicity pathways and underlying molecular mechanisms.

Methods: A structured literature review was conducted using peer-reviewed databases. Studies involving hepatic (HepG2, HepaRG, PRH), neuronal (SH-SY5Y, primary cortical cultures, NSPCs), and cardiac (hiPSC-derived cardiomyocytes) models were included. Endpoints assessed comprised cell viability, mitochondrial function, oxidative stress, apoptosis, genotoxicity, electrophysiology, and enzyme-mediated metabolism.

Results: Cytotoxic effects were broadly observed across experimental models in a concentration- and time-dependent manner. Hepatotoxicity was consistently observed for MDPV, methylone, mephedrone, 4-MEC, naphyrone, buphedrone, and 3,4-DMMC, with primary rat hepatocytes showing greater sensitivity than HepG2 and HepaRG cells. Naphyrone and 3,4-DMMC exhibited the highest hepatotoxic potency, inducing effects at lower concentrations than most analogues.

Mitochondrial dysfunction was identified as a central mechanism, characterized by disruption of mitochondrial membrane potential, inhibition of electron transport chain complexes I–III, and ATP depletion. Naphyrone and MDPV markedly impaired oxidative phosphorylation, while 3,4-DMMC and butylone induced concentration-dependent mitochondrial hyperpolarization and depolarization. These effects were accompanied by oxidative stress, including increased ROS/RNS production, glutathione depletion, and elevated lactate formation, with naphyrone showing the strongest pro-oxidant activity.

Neurotoxicity was characterized by reduced neuronal viability, synaptic dysfunction, and impaired network firing. α-PVP and MDPV showed the strongest neurotoxic effects, while N-ethylhexedrone and 4-FMC also exhibited high cytotoxicity in SH-SY5Y cells. In primary neuronal cultures, α-PHP and 3,4-MDPHP induced morphological damage, reduced proliferation, and increased ROS formation. 3-CBC, 4-CBC, and 3-Cl-DEC further contributed to mitochondrial depolarization and oxidative stress, with additional acetylcholinesterase inhibition observed. Further contributing to neurotoxic risk through monoaminergic mechanisms, 3F-NEB and α-PiHP displayed strong dopaminergic activity and reinforcing effects, with chronic exposure associated with elevated ΔFosB expression. 3,4-MD-α-PHP, 4-Cl-α-PPP, and α-PiHP showed abuse-related profiles

comparable to methamphetamine and cocaine via potent DAT and NET inhibition. Mephedrone and 4-CMC acted as non-selective monoamine transporter substrates, whereas 4-Cl-α-PPP and 4-CEC displayed selective transporter inhibition profiles.

Cardiotoxicity was evidenced by MDPV- and α-PVP-induced electrophysiological alterations in cardiomyocytes, including changes in beat rate, spike amplitude, and field potential duration, with MDPV showing higher potency. Genotoxic effects included dose-dependent DNA double-strand breaks, further modulated by NAT2 polymorphisms affecting metabolic activation and DNA damage susceptibility.

Discussion: Synthetic cathinones exert multi-system toxicity driven by mitochondrial dysfunction, oxidative stress, monoaminergic transporter interactions and genetic susceptibility, highlighting their high toxicological risk across biological systems. The differential potency observed across analogues underscores the need for structure-specific risk assessment rather than class-wide regulatory approaches. The rapid structural diversification of synthetic cathinones continues to outpace toxicological characterization such as N-ethylnorpentedrone, in-vitro organ-specific toxicity endpoints and structure–activity relationships governing oxidative and genotoxic potential remain to be systematically established.

Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

P-333

Cytotoxic Effects of MDMB-4EN-PINACA and 5F-ADB in HepG2, SH-SY5Y, and AC16 Cell Lines

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Abstract

Introduction: Synthetic cannabinoids (SCs) constitute the largest evolving groups of new psychoactive substances, with indazole-carboxamide derivatives, including 5F-ADB and MDMB-4en-PINACA, increasingly detected worldwide. Despite their association with severe acute toxicity and frequent involvement in postmortem cases, organ-specific cytotoxicity data remain largely absent from literature. This gap critically limits the interpretation of forensic cases and impedes evidence-based risk assessments.

Objectives: This study aimed to evaluate the preliminary cytotoxic effects of 5F-ADB and MDMB-4en-PINACA in human hepatic (HepG2) and cardiomyocyte (AC16) cell lines across a concentration range of 1–150 µM over different exposure durations with determining IC_{50} values. Neuronal cytotoxicity studies using SH-SY5Y cells are ongoing and will be incorporated into the final dataset.

Methods: This study was supported by the I.U.-Cerrahpaşa Scientific Research Projects Coordination Unit (Project No: TOA-2025-38589). HepG2 and AC16 cells were exposed to 5F-ADB and MDMB-4en-PINACA at concentrations of 1, 5, 10, 25, 50, 100, and 150 µM for 24, 48, and 72 hours. Cell viability was assessed using the MTT reduction assay and optical density values were normalized to vehicle-treated controls. Preliminary IC_{50} values were calculated using nonlinear regression with a four-parameter logistic model in GraphPad Prism (v.11) flanking the 50% inhibition threshold within the tested range; conditions where viability did not reach this threshold were reported as IC_{50} >150 µM, and conditions exhibiting non-monotonic dose-response behavior were not assigned IC_{50} values.

Results: Both SCs induced generally concentration- and time-dependent reductions in cell viability in both cell lines. In HepG2 cells, 5F-ADB demonstrated pronounced time-dependent cytotoxicity. Viability remained >50% inhibition threshold across all concentrations at 24h (IC_{50} >150 µM), followed by a marked increase in potency at 48h, with IC_{50} ~25–50 µM, which was sustained at 72h (IC_{50} ~42 µM). MDMB-4en-PINACA exhibited a comparatively gradual cytotoxic profile in HepG2 cells, with IC_{50} values of >150 µM at 24h and ~117 µM at 48h; for 72h yielding IC_{50} of ~100 µM.

In AC16 cardiomyocytes, two substances demonstrated distinct temporal cytotoxicity patterns. For MDMB-4en-PINACA, cytotoxic effects were not apparent at 24h (IC_{50} >150 µM), whereas progressive toxicity was observed at longer exposure durations, with IC_{50} estimated between 25–50 µM at 48h (~30 µM) and ~33 µM at 72h. For 5F-ADB, cytotoxicity was observed earlier, with

IC_{50} of ~75 µM at 48h and ~57 µM at 72h; IC_{50} could not be reliably determined at 24h due to a non-monotonic dose-response pattern within the tested concentrations.

Discussion: These preliminary findings demonstrate that 5F-ADB and MDMB-4en-PINACA exert cytotoxic effects time-dependently for both cell types, with distinct organ-specific and temporal potency profiles. The sharp decline in HepG2 viability emphasized more vulnerability to 5F-ADB. In AC16, 5F-ADB showed earlier initiation of cytotoxicity, while MDMB-4en-PINACA demonstrated greater potency at prolonged exposure durations, particularly at 72h, indicating cardiotoxic action warranting further investigation. Ongoing SH-SY5Y studies will further delineate the neurotoxic profiles. Collectively, these data contribute to the experimental characterization of up-to-date SCs and support the need for substance-specific, multi-organ risk assessment frameworks in both forensic and clinical toxicology contexts.

Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

P-334

Is Xylazine Being Replaced by Medetomidine in Fentanyl Cases in Alabama?

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Abstract

Introduction: Additives and adulterants are often found in clandestine drug manufacturing. These may be added to increase weight/volume or to enhance the effects of a drug. Xylazine, a veterinary sedative, is a common adulterant detected in casework. It is frequently identified in fentanyl-positive cases, suggesting intentional adulteration of illicit fentanyl. Recently, another veterinary sedative, medetomidine, has emerged as a new adulterant to fentanyl.

Medetomidine is an α_2-adrenergic agonist not approved for human use by the Federal Drug Administration. Medetomidine is poorly studied in humans and is not reversible by naloxone. It is estimated to be 200-times more potent than xylazine. Despite limited information on human effects, a 2024 cluster of overdose cases in Chicago, Illinois, provided some insights. Reported signs and symptoms include bradycardia, altered mental status, pinpoint pupils, hypoxemia and hypertension.

Objectives: To evaluate toxicology data at the Alabama Department of Forensic Sciences (ADFS) from ante-mortem and post-mortem cases to determine the prevalence of xylazine and medetomidine in fentanyl-positive blood cases between October 2023 and April 2026 (n=1474).

Methods: Medetomidine was optimized on an Agilent Liquid Chromatograph (1260 or 1290), Tandem Mass Spectrometer (6460, 6470, or Ultivo) (LC/MS/MS), and identification was based on retention time and multiple reaction monitoring transitions with acceptable ion ratios. Previous data collected on an Agilent 1290 Liquid Chromatograph and 6545 Quadrupole Time-of-Flight (Q-ToF) were also included. Medetomidine detection was limited to the latter part of the study following method implementation. Xylazine was validated on the LC/MS/MS method in October 2023 and on the Q-ToF in July 2024.

Results: From 2023 to 2026, ADFS reported 1,474 fentanyl-positive cases. A total of 526 fentanyl-positive cases containing xylazine were identified. Annual distribution and prevalence was as follows: 2023 (n = 19, 3.4%), 2024 (n = 312, 59.3%), 2025 (n = 159, 30.0%), and 2026 (n = 36, 6.8%). The subjects were predominantly males (74%) and disproportionately white individuals (80%). Medetomidine was added to the LC/MS/MS method for monitoring purposes in March 2026, however, it was validated on the Q-ToF method in June 2025. Medetomidine was identified in five cases by Q-ToF, and in 14 cases by LC/MS/MS.

Discussion: While xylazine was analyzed in all fentanyl-positive cases, medetomidine was only included in the analysis in the final two months of the study. Over the study period, xylazine was frequently detected in casework at ADFS, with data suggesting that approximately 36% of the fentanyl users in Alabama were receiving xylazine-adulterated illicit fentanyl. A steady decline is evident, with an approximate 50% decrease observed between 2024 and 2025. Preliminary 2026 data suggest a continued decline. The abundance of xylazine cases throughout the state suggests widespread usage, predominantly in the northern part of the state. Medetomidine has

been identified in a limited, but increasing number of cases. Since implementation of LC/MS/MS testing, 11 cases of medetomidine have been co-identified with xylazine and fentanyl, while three cases contain only medetomidine and fentanyl. It is unclear if medetomidine will replace xylazine or become a co-adulterant; future data will elucidate these trends.

Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

P-335

Carfentanil: You Don't Know What You Can't See. Are You Seeing The Whole Picture?

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Abstract

Introduction: Carfentanil is an ultra-potent, fentanyl analog that remains prevalent in opioid overdose cases in Alberta, Canada. Due to its potency, carfentanil is present in biological specimens at exceedingly low concentrations which presents analytical challenges for detection and quantification. Currently there is no ANSI/ASB recommended analytical sensitivity for carfentanil; however, considering its estimated potency, i.e., ~100 times greater than fentanyl, one could use the ANSI/ASB recommendation of 1 ng/mL for fentanyl as a basis for proposing an appropriate sensitivity recommendation for carfentanil, i.e., 1/100th of 1 ng/mL = 0.01 ng/mL.

Many conventional screening methods lack sufficient sensitivity, which can lead to false-negative analytical results that under-report the presence and prevalence of carfentanil. The reported occurrence and impact of this drug in drug-related deaths may be falsely low across jurisdictions. These challenges highlight the need for highly sensitive analytical screening and confirmatory techniques which are capable of reliably detecting and quantifying carfentanil in biological matrices, thereby improving the reliability of forensic toxicological data and subsequent interpretations in drug-related deaths.

Objectives: To demonstrate the need for a highly sensitive screening technique which prevents significant under-reporting of the prevalence of carfentanil. This presentation demonstrates that the required sensitivity for carfentanil is considerably lower than recommended for other opioid drugs and much lower than many current, conventional screening methods, which could under-report a significant majority of positive cases. Concentrations of carfentanil (in ligated femoral blood) are presented from drug-fatality casework.

Methods: A robust, sensitive liquid chromatography tandem mass spectrometry (LC-MS/MS) screening method was developed to screen for the presence of carfentanil in all cases. Confirmation and quantification of carfentanil was undertaken using a validated LC-MS/MS method. The screening method uses an acetonitrile/MeOH precipitation with 0.1 mL blood and 0.2 mL reagent. For enhanced scope and sensitivity, the extract is analyzed by quadrupole time-of-flight (QTOF, Agilent 6545) and triple-quadrupole (QQQ, Agilent 6475). Carfentanil detection on the QQQ screen has a limit of detection (LOD) of 0.01 ng/mL (qualifier within 20%). Quantitative analysis of carfentanil was performed on an Agilent 6460 QQQ, utilizing a SPE extraction. The lowest calibrator was 0.02 ng/mL (range 0.02 – 2 ng/mL), with a reporting limit of 0.01 ng/mL.

Results:

Prevalence of Carfentanil	
Year	**No. of Cases (> 0.02 ng/mL)**
2021	384
2022	385
2023	237
2024	83
2025	628

Impact of Screening Sensitivity - 2025 Data	
Cut-Off (ng/mL)	**Cases Missed (% Cumulative)**
0.02	3
0.05	8
0.1	20
0.2	45
0.5	75

Discussion: In our laboratory, all cases requiring drug screening (~4800 cases in 2025) are screened for carfentanil, as described earlier. It is critical that drug screening techniques have sufficient sensitivity to reliably identify carfentanil and trigger follow-up confirmatory and quantitative analysis. LCQTOF-MS screening with a reported LOD of 0.5 ng/mL would not identify approximately 75% of carfentanil positive cases based on our dataset. This LOD is clearly unsuitable for application in toxicology casework. Although targeted drug screening with sensitivity at 0.05 ng/mL LOD shows improved performance, this would still under-report approximately 8% of carfentanil positive cases based on our data. As such, we recommend a targeted screening sensitivity of 0.02 ng/mL or lower, for confident assessment of carfentanil positivity in postmortem blood.

Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

P-336

From Metabolite to Misuse: O-Desmethyltramadol in Two Mixed-Drug Fatalities and Seized Materials

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Abstract

Introduction: O-desmethyltramadol (O-DSMT) is the principal active metabolite of the commonly prescribed synthetic opioid analgesic tramadol. It has circulated as a new psychoactive substance (NPS) on the European drug market since approximately 2008. O-DSMT is not approved for medical use and shows a substantially higher μ-opioid receptor affinity than tramadol, consistent with its greater analgesic potency. One study reported nine deaths involving O-DSMT mixed with other substances, though published data on O-DSMT-related fatalities remain scarce.

Objectives: We present two independent postmortem cases from 2024 and 2025 involving multi-drug use including O-DSMT. The drugs were analyzed in the available biological matrices (femoral blood, urine, liver, stomach content and hair). Additionally, two seized materials were examined; tablets related to one of the postmortem cases, and another seized crystalline powder.

Methods: Crime scene investigations were followed by forensic autopsy and sample collection. Postmortem samples were analyzed using our systematic toxicological analysis (STA) protocol and quantitative analytical methods. O-DSMT concentrations were measured in the matrices using a validated method based on liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS). The presence and concentrations of other drugs were determined using validated methods based on LC-MS/MS and gas chromatography (GC) coupled to MS. The seized materials were analyzed using LC-diode array detection, GC-MS and LC-quadrupole time of flight (QTOF)-MS for identification.

Results: Both decedents were men in their late twenties to early thirties. In case 1, CPR was unsuccessful. In case 2, one victim was found deceased while a second, unconscious individual was hospitalized. In both cases, multiple drug use was confirmed. In case 1, O-DSMT blood and urine concentrations were 195 ng/mL and > 1000 ng/mL, respectively. In case 2, blood and urine concentrations exceeded 1000 ng/mL, and O-DSMT was detected in liver. In both cases, O-DSMT was detected in stomach content, indicating recent oral ingestion. Neither tramadol nor N-desmethyltramadol was detected in any of the matrices, indicating direct O-DSMT ingestion. In case 2, hair samples were available as well, and a hair analysis is carried out to evaluate historical use of O-DSMT. Both seized materials contained O-DSMT without tramadol. There were indications that the substances had been obtained through purchase from an easily accessible website.

Discussion: In case 1, severe interactions between multiple substances (i.e., designer benzodiazepines and psychoactive medication), rather than a single toxic dose may explain death, given the relatively low O-DSMT blood concentration. Blood levels of tramadol > 1000 ng/mL are considered potentially fatal. Although established fatal concentration ranges for O-DSMT remain limited, the high blood concentration in case 2, together with co-intoxication with bromazolam, supports significant toxicological contribution. The combination likely caused severe sedation and a prolonged terminal phase, consistent with the high urine concentration. Postmortem redistribution, well documented for tramadol, may also affect O-DSMT, potentially elevating measured blood concentrations. In both cases, urine concentrations of O-DSMT exceeded those typically reported in therapeutic tramadol users (100-1000 ng/mL). Additionally, its presence in stomach contents suggests recent use as well. These cases underscore the need for greater awareness and stricter regulations of easily accessible websites selling these NPS.

Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

P-337

A Five-Year Longitudinal Evaluation of the Behavioral and Toxicological Factors Defining the Trauma-Toxicity Continuum in Methamphetamine Fatalities

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Abstract

Introduction: Methamphetamine ("shabu") is an emerging medico-legal concern in Western Saudi Arabia. In our earlier Jeddah surveillance, the absolute number of methamphetamine-positive deaths rose more than five-fold between 2016 and 2018, but whether this represented a transient spike or the leading edge of a sustained pattern was unresolved, and matrix-specific interpretive data for postmortem casework in this population were lacking.

Objectives: To characterize the temporal trajectory, demographic and toxicological profile, and multimatrix concordance of methamphetamine-positive medico-legal deaths in Jeddah over a five-year window spanning the COVID-19 era.

Methods: We retrospectively reviewed all medico-legal deaths investigated in Jeddah, Saudi Arabia (2018-2022) that were positive for methamphetamine and/or its metabolite amphetamine in at least one matrix by confirmatory LC–MS/MS (n = 90). Specimens (peripheral [subclavian] blood, vitreous humor, gastric contents, bile, urine, liver, and additional tissues) underwent immunoassay and general-unknown screening followed by confirmatory quantification by LC–MS/MS, validated per ANSI/ASB Standard 036 (LOD 0.3 ng/mL; LOQ 1.0 ng/mL; linear 1-1000 ng/mL). Amphetamine was quantified alongside methamphetamine; comprehensive screening also covered opioids, cannabinoids, benzodiazepines, cocaine, ketamine, ethanol, and other CNS-active drugs. Peripheral (subclavian) sampling with cross-matrix concordance was used to mitigate postmortem redistribution. Manner of death was adjudicated from scene, autopsy, and toxicology. Group comparisons used Kruskal–Wallis tests; matrix-pair concordance used Spearman's rho.

Results: Absolute case counts rose from 10 (2018) to 27 (2022), a 2.7-fold increase, with 56.7% (51/90) concentrated in 2021-2022. Decedents were predominantly aged 21-40 years; deaths occurred mainly in home/residential settings (44/90, 48.9%) and outdoor/public locations (31/90, 34.4%). Manner of death was accidental in 65.6% (59/90), suicide in 17.8% (16/90), and homicide in 16.7% (15/90); violent deaths comprised 34.4%. Blood concentrations overlapped broadly across manner of death (methamphetamine median [IQR]: accidental 260 [116-664], suicide 261 [145-515], homicide 533 [168-816] ng/mL; $H = 0.66$, $p = 0.718$; amphetamine $p = 0.558$). Across the defined COVID-19 intervals [see Part C for period definition], median blood methamphetamine was statistically comparable (2018: ~300 [IQR 220-540] ng/mL, n = 10; 2019-2020: ~230 [150-460] ng/mL, n = 29; 2021-2022: ~340 [140-980] ng/mL, n = 51; $p > 0.05$), indicating the post-2020 resurgence reflects a higher number of deaths rather than higher per-case exposure. Toxicology profiles partitioned into methamphetamine-only (38.9%), polydrug

intoxication (32.2%), and methamphetamine with non-contributory co-detections (28.9%). Multimatrix concordance with peripheral blood was strongest for vitreous humor (rho = 0.845; n = 69), gastric contents (rho = 0.800; n = 28), and bile (rho = 0.729; n = 23).

Discussion: Methamphetamine-positive deaths in Jeddah are sustained and increasing, driven by case frequency rather than escalating per-case exposure, and manner of death cannot be inferred from blood concentration alone. Vitreous-, gastric-, and bile-to-blood concordance values provide quantitative interpretive support when peripheral blood is unavailable or compromised, and argue for routine multimatrix sampling and integrated case interpretation in regional forensic practice. These figures are absolute forensic case counts, not population-adjusted incidence.

Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

P-338

When Ecstasy Isn't So Ecstatic: A Possible Case of Suicide by MDMA

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Abstract

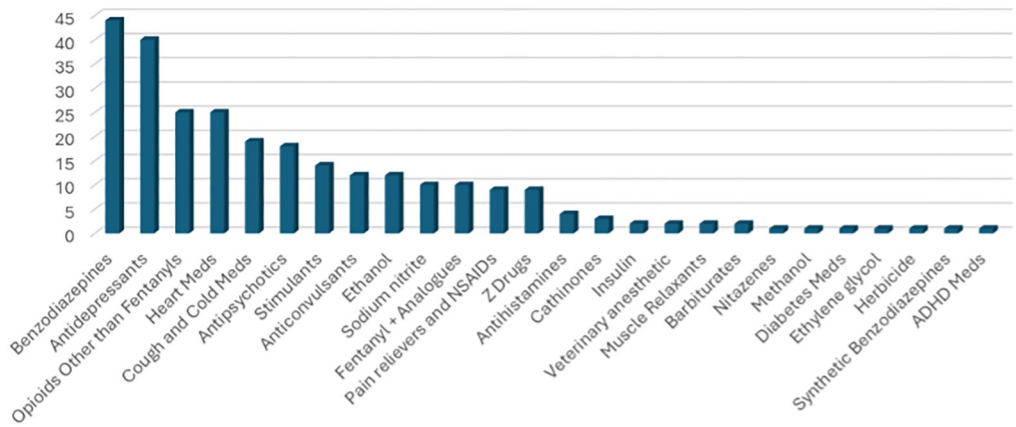
Introduction: Suicide by toxic ingestion is a common manner of death encountered in postmortem death investigation. Toxicological findings alone are often insufficient for determining the manner of death and must be interpreted in the context of investigative findings, autopsy results, scene circumstances, and social and medical history. Distinguishing between suicide and accident can be particularly challenging, especially in cases involving polysubstance use or atypical drug patterns.

Objectives: This presentation aims to characterize substances involved in suicidal overdose cases at the Miami-Dade County Medical Examiner Department (MDME) and to summarize a complex case that contrasts with typical patterns, highlighting the importance of integrating all aspects of the investigation to determine manner of death.

Methods: A retrospective study was performed of cases over a five-year period in which the manner of death was certified as suicide due to toxic ingestion. Exclusions included multi-modality suicides, inhalation deaths, and cases in which toxic substances were not listed in the cause of death. Substances listed in the cause of death were tabulated. Futher, a representative complex case was evaluated, including demographic information, investigative findings, medical and social history, scene documentation, and toxicological results.

Results: From January 1, 2021 through April 27, 2026 at the MDME, 126 suicidal overdose cases meeting inclusion criteria were identified. Benzodiazepines and antidepressants were the most frequently encountered substances (Table 1), and many cases involved multiple substances. Overall, the findings reflect common patterns of suicidal ingestion involving central nervous system depressants and mixed drug use.

Table 1: Substances Presence in Suicidal Ingestion at the MDME



In contrast to these patterns, a 34-year-old white female was found deceased at home with a complex toxicology profile. Ethanol was present at 0.038% in iliac vein blood and 0.057% in ocular fluid. Additional findings included cocaine and metabolites, methamphetamine, diphenhydramine, hydroxyzine, oxymetazoline, trazodone, 3,4-methylenedioxy-N-propylamphetamine, ketamine, methylone, lysergic acid diethylamide (LSD), and 2-oxo-3-hydroxy-LSD. Notably, methylenedioxy methamphetamine (MDMA) and methylenedioxy amphetamine (MDA) were present at 37 and 0.935 mg/L, respectively. These substances are not commonly associated with suicidal ingestion. However, two handwritten notes recovered at the scene expressing her love and saying goodbye to her family supported a classification of suicide. Despite this, the decedent's parents raised concerns regarding abuse by her boyfriend and that the decedent may have been coerced into ingesting substances.

Discussion: Substances involved in suicidal ingestion in Miami-Dade County vary widely but generally follow recognizable patters. Although this case was not included in the retrospective study and remains pending final classification, it illustrates how deviation from these patterns can complicate manner of death determination. Even in the presence of high drug concentrations and apparent suicidal ideations, alternative scenarios such as accidental ingestion or coercion must be considered. These findings underscore the importance of a comprehensive, multidisciplinary approach in forensic casework, where toxicology serves as only one component of a broader investigative framework and where the final manner of death may remain uncertain.

Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

P-339

Forensic Toxicological Interpretation of Fatal Overdose Involving Fentanyl and Multiple Co-Intoxicants.

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Abstract

Introduction: Deaths involving illicit and nonmedical benzodiazepine use are frequently complicated by co-exposure to opioids, antihistamines, cocaine-related substances, and other central nervous system depressants. This case involves a 29-year-old man with a reported history of drug and alcohol abuse. According to the investigative history, he visited the beach and reportedly used alprazolam, locally known as “Pali.” After returning home, he went to bed and was later found deceased.

Objectives: To describe the postmortem toxicological findings in a young adult male with a history of substance abuse and to evaluate the fentanyl, alprazolam, diphenhydramine, benzoylecgonine and morphine co-exposure, drug interaction and contribution to fatal intoxication.

Methods: Drug analytes were extracted from postmortem cardiac blood using solid-phase extraction. Initial toxicological screening was performed by enzyme-linked immunosorbent assay, followed by confirmatory analysis using liquid chromatography–time-of-flight mass spectrometry, liquid chromatography–tandem mass spectrometry and gas chromatography–mass spectrometry. The toxicological findings were interpreted in the context of the investigative history and the reported use of alprazolam prior to death.

Results: Cardiac blood analysis identified fentanyl at 24 ng/mL and norfentanyl at 5.2 ng/mL. 4-ANPP was also detected qualitatively, supporting fentanyl exposure or association with fentanyl-related compounds. Alprazolam was present at 40 ng/mL, a concentration consistent with pharmacologically significant benzodiazepine exposure. Additional findings included diphenhydramine at 580 ng/mL, benzoylecgonine at 470 ng/mL, free morphine at 39 ng/mL, acetaminophen at 9,600 ng/mL and qualitative detections of caffeine, nicotine, cotinine, lidocaine, and monoethylglycinexylidide. The fentanyl concentration was within a range associated with fatal intoxication, particularly in the presence of other central nervous system depressants. Diphenhydramine was detected at a concentration exceeding typical therapeutic levels and may have contributed to sedation and toxicodynamic interaction.

Discussion: The circumstances of death and toxicological profile support a fatal mixed-drug intoxication, with fentanyl likely serving as the principal driver of respiratory depression. Alprazolam use is consistent with the detection of alprazolam in cardiac blood and is forensically relevant because benzodiazepines can potentiate opioid induced sedation and respiratory compromise. The presence of diphenhydramine adds another sedating agent, while free morphine indicates additional opioid exposure. Benzoylecgonine supports recent or prior cocaine use, reflecting polysubstance abuse in the setting of known drug and alcohol abuse. Lidocaine and its metabolite suggest medical intervention or drug-adulterant exposure and should be interpreted with case context.

Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

P-340

Drug-Involved Pediatric Deaths in Georgia: A Forensic Toxicology Perspective

Denise Carter, Ashley Garrish, Dr. Lora Darrisaw, Dr. Michelle Di Marco, Vanessa Schmidt, Stephanie Marino, Twyla Coverson

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Abstract

Introduction: Pediatric drug-involved deaths are uncommon but carry significant implications for forensic toxicology, public health, and child safety. Interpretation of toxicological findings in children is particularly complex due to developmental physiology, pharmacokinetics, and varied exposure pathways. In recent years, increased availability of illicit drugs especially synthetic opioids has raised concern regarding both accidental and intentional exposures among minors.

Objectives: To characterize temporal trends, substances involved, and demographic patterns in pediatric drug-related deaths in Georgia from 2015 to 2024, and to assess the role of forensic toxicology in identifying emerging drug threats and informing prevention strategies.

Methods: A retrospective case review was conducted using data from the Georgia Bureau of Investigation (GBI) Medical Examiner's Pediatric Unit. Cases included decedents aged ≤17 years with drug involvement between 2015 and 2024. Toxicological analyses were performed using liquid chromatography-tandem mass spectrometry (LC-MS/MS), gas chromatography-mass spectrometry (GC-MS), and liquid chromatography-high resolution tandem mass spectrometry (LC-HRMS/MS) following validated extraction protocols. Variables analyzed included age, sex, race, manner of death, and detected substances. Descriptive statistical methods were used to assess trends and distributions over time.

Results: A total of 99 pediatric drug-involved deaths were identified during the study period. Case frequency increased notably between 2020 and 2022, followed by a modest decline in subsequent years. Adolescents aged 13–17 years accounted for the majority of cases. Accidental and suicidal manners of death were most commonly reported. Fentanyl and methamphetamine were the most frequently detected substances across the dataset. Multiple cases also involved over-the-counter medications, including diphenhydramine and dextromethorphan. Overall, findings demonstrate a shift toward more potent and high-risk substances over time, with synthetic opioids increasingly represented in recent years.

Discussion: The observed increase in pediatric drug-related deaths during the COVID-19 pandemic likely reflects a combination of social and environmental factors, including reduced supervision, increased isolation, and greater access to both prescription and illicit substances. The predominance of fentanyl is particularly concerning due to its high potency and frequent presence in counterfeit pills, significantly increasing the risk of unintentional overdose among adolescents.

The involvement of commonly available over-the-counter medications highlights an additional and often under-recognized source of pediatric risk within household environments. Although the overall number of cases remains relatively low, the upward trend and evolving drug landscape underscore the need for continued surveillance and targeted prevention efforts.

Forensic toxicology plays a critical role in identifying causative agents and contributing to public health surveillance systems. These findings emphasize the importance of collaboration between forensic laboratories, medical examiners, and public health agencies to support education initiatives, safe storage practices, and early intervention strategies aimed at reducing pediatric drug exposure and mortality.

Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

P-341

Low-Concentration Acetone as a Negative Indicator for Significantly Elevated BHB in Postmortem Casework

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Abstract

Introduction: Beta-hydroxybutyrate (BHB) is widely considered the best indicator for ketosis, with blood levels greater than 250 mg/L considered pathologically significant. Acetone can be formed from the precursor of BHB (i.e., acetoacetate) and is a commonly detected analyte in postmortem casework, making it a less-reliable indicator for ketosis than BHB itself. Historically, the majority of acetone analytical methods have detection (or reporting) limits in the range of 2-4 mg/dL in blood. However, this concentration of acetone is not considered a reliable indicator for the absence of life-threatening ketosis.

Objectives: In the data presented, the quantitative analytical method for acetone permits a reporting limit of 0.5 mg/dL. Acetone and BHB results from over 900 postmortem cases from 2021-2025 will be compared, with particular focus on acetone results below 2 mg/dL.

Methods: Essential to this study is a combination of two validated methods: a) a highly specific quantitative assay for BHB (LLOQ 50 mg/L) and b) a method capable of quantifying acetone with a LLOQ of 1 mg/dL (reporting limit of 0.5 mg/dL). The BHB assay employs the specificity of GC-MS/MS, using GHB-D_6 as an internal standard. Briefly, 0.1 mL of blood was protein precipitated using acetonitrile; the supernatant was dried down and derivatized with BSTFA (with 1% TMCS). Derivatized extracts were analysed using an Agilent 7000A GC-QQQ. For acetone analysis, 0.1 mL of blood was diluted with 2.0 M NaCl containing two internal standards (N-propanol and tert-butanol). A dual column HS-GC-FID was used to analyze for acetone and other volatiles (i.e., ethanol, methanol, and isopropanol). Calibrators and quality control samples were prepared in-house from certified reference materials, allowing customized dynamic ranges for all compounds. The system was calibrated daily, with 6 freshly prepared acetone calibrators at 1, 2.5, 5, 10, 30, and 50 mg/dL.

Results:

	# Cases		
Acetone (mg/dL)	**Total**	**BHB > 250 mg/L**	**BHB > 200 mg/L**
ND	36	0	0
0.5 to < 1.0	32	0	0
1.0 to 1.3	63	0	1
1.4 to < 2.0	71	10	15
2.0 to < 3.0	128	19	31

Discussion: All cases requiring toxicology testing were analyzed for acetone, ethanol, isopropanol, and methanol. The previous protocol in our laboratory was to consider reflex testing for BHB if the acetone concentration exceeded 2 mg/dL. Recently, an increasing number of clients (i.e., medical examiners) have requested BHB analysis at file initiation, based on the case history and autopsy observations. As such, the dataset has expanded to include BHB results with acetone less than 2 mg/dL. The prevalence of BHB was considered at concentrations of >250 mg/L and at >200 mg/L (i.e., -20% to account for a range of possible measurement error).

The data presented suggests that an acetone concentration of less than 1 mg/dL in postmortem blood could be a reliable indicator that BHB is not significantly elevated and negate the need for BHB quantitative analysis.

Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

P-342

The Rise of Nitazenes: A Scottish Perspective

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Abstract

Introduction: Scotland has the highest rate of drug-related deaths in Europe, with 19.1 drug-misuse deaths per 100,000 people in 2024 (the last year for which statistics are complete). The main contributor towards these deaths has historically been opioids (80% in 2024), comprising mostly heroin/morphine and the opioid substitute methadone.

In April 2022, the Taliban banned production of opium in Afghanistan, leading to a reported 93% reduction in opium production. Traditionally, heroin available on the European market had come from this region, and as a result of this ban the availability and purity of heroin in Scotland were reduced.

Enter nitazenes - highly potent opioids, initially developed in the 1950s for pharmaceutical research. In 2019 the first nitazene was reported to the UNODC (isotonitazene), and as of 2024 another 34 substances have emerged.

While fentalogues have never featured significantly in Scottish post-mortem toxicology casework, particularly compared to other countries, the vacuum left by the heroin shortage meant that people who use drugs were left seeking an alternative. On top of this, there have been reports of nitazene detection in drugs sold as oxycodone and benzodiazepines so people who use these drugs may be unaware of what they were taking.

Objectives: The objective of this work was to analyse trends in drug-related deaths in Scotland with a focus on “traditional” opioids such as heroin and methadone, and “novel” opioids such as nitazenes.

Methods: Data from the National Records of Scotland’s Drug-Related Deaths in Scotland reports were used to make comparisons between number of deaths (as a percentage of inclusions in all drug-related deaths) per year by drug/drug type. Further data were extracted from the SPA Forensic Services in-house database to more closely examine the nature and trends of nitazene use in more recent years.

Results: Between 2019 and 2024, the percentage of heroin/morphine-related deaths in Scotland fell from 51% to 31%. Nitazenes were first included in a cause of death in Scotland in 2022 (N=1, protonitazene). By 2024, 7% of drug-related deaths in Scotland implicated one or more nitazenes. For additional context, the percentage of methadone-related deaths remained fairly consistent, with a slight reduction over the same time period from 44% to 41%.

Discussion: While heroin use has a long history of causing the a significant proportion of drug-related deaths in Scotland, the reduction in the availability of this substance in the market has created a vacuum for other drugs to fill. What is of concern is the emergence of the use of the highly potent nitazenes. First detected in post-mortem casework in Scotland in 2022, several nitazene drugs have now been included within causes of death.

SPA Forensic Services conducts the post-mortem toxicology casework for the majority of Scotland, and as such were required to launch a rapid response to the threat of nitazenes within the drugs market. Testing paraphernalia seized from deaths and near-fatal overdoses allowed detection of the compounds involved, and the development of a targeted analytical method for relevant nitazene drugs in post-mortem blood, urine and vitreous humour.

Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

P-343

Screening, Confirmation, Prevalence, and Fatality of Digoxin in Finland in 2012-2025

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Abstract

Introduction: Digoxin, a cardiotonic glycoside derived from Digitalis lanata, is widely prescribed for the treatment of congestive heart failure and atrial fibrillation. However, it has a narrow therapeutic index and a significant risk of toxicity. Post mortem (PM) redistribution complicates the interpretation of digoxin concentrations, necessitating careful correlation with clinical history and case circumstances. In Finland annual deaths of 18–36 are attributed to digoxin toxicity (2012–2020), higher than other narrow therapeutic index drugs like lithium or warfarin. Many cases are due to chronic accumulation rather than acute overdose.

Objectives: Primary objective was to evaluate analytical methods used for digoxin detection in PM blood samples in Finland during 2012 to 2025. Secondary objective was to assess the prevalence of digoxin detection in forensic autopsy cases and the occurrence of fatal digoxin intoxications in Finland.

Methods: Analysis was initially performed using immunoassays, which were later supplemented and replaced by LC MS/MS to improve analytical specificity. Data were collected from all medico legal forensic autopsies performed in Finland between 2012 and 2025. Digoxin screening and quantification were initially performed using immunoassays (Siemens Immulite 1000, Immulite 2000, and Advia Centaur). Positive findings and selected samples were later confirmed using LC MS/MS (Sciex 4000 QTrap and Agilent 6475 instruments). In total, more than 34,000 analyses were conducted.

Results: Immunoassay concentrations were approximately 25% lower than those obtained using LC-MS/MS, with differences ranging from 82% to 53%. In 26 cases, the immunoassay result was higher than that obtained by LC-MS/MS, whereas in the remaining 208 cases it was equal to or lower. Concentrations were similar between the two methods, showing no significant differences.

Discussion: Immunological screening methods resulted in a high number of analyses but a relatively low positive rate. Comparative testing performed between 2019 and 2024 demonstrated that immunoassays exhibited high specificity but systematically underestimated digoxin concentrations by approximately 25% compared with LC-MS/MS. Digoxin levels in PM heart blood can be up to three times higher than ante-mortem peripheral blood in the same patient. This is due to diffusion from myocardium and other tissues after death. Post-mortem redistribution means that high post-mortem levels do not automatically prove toxic ante-mortem levels, therefor clinical context and case history are essential. Suggested approach enhances resource efficiency and strengthens the reliability of toxicological investigations, particularly in elderly populations where digoxin use and risk of intoxication are most prevalent. Fatal intoxications (36 cases) occurred predominantly in elderly patients, with mean concentrations far exceeding therapeutic levels.

Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

P-344

Fatal Mixed-Drug Intoxication Involving Methadone, Fluoxetine, Tramadol, and Multiple Sedative Co-Exposures

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Abstract

Introduction: Mixed-drug intoxication remains a major challenge in forensic toxicology because drug concentrations must be interpreted within the context of pharmacological overlap, tolerance, postmortem redistribution, medical history, and investigative findings. This case involves a 37-year-old woman reportedly experiencing cold-like symptoms before death. Family members reported multiple prior suicide attempts, recurrent overmedication, and excessive daytime sleeping. She was later found deceased in bed by relatives.

Objectives: Characterize fatal mixed-drug intoxication involving multiple prescribed psychoactive substances and evaluate the toxicological significance of combined opioid, antidepressant, anticonvulsant, benzodiazepine-related, and barbiturate exposure in the context of prior suicidal behavior and recurrent overmedication.

Methods: Postmortem cardiac blood and gastric fluid were analyzed using enzyme-linked immunosorbent assay, gas chromatography-mass spectrometry, liquid chromatography-time-of-flight mass spectrometry, and liquid chromatography–tandem mass spectrometry. Peripheral blood was unavailable, representing a major limitation because cardiac blood concentrations are susceptible to postmortem redistribution. Total gastric fluid volume was not recorded, preventing calculation of total drug mass in gastric contents. Quantitative and qualitative findings were interpreted using investigative history and published therapeutic, toxic, and fatal concentration ranges.

Results: Cardiac blood analysis identified methadone at 180 ng/mL and its primary metabolite, EDDP, at 160 ng/mL, indicating methadone exposure with metabolism. Additional cardiac blood findings included fluoxetine at 1,500 ng/mL and norfluoxetine at 1,500 ng/mL; 7-aminoclonazepam at 84 ng/mL; tramadol at 300 ng/mL; O-desmethyltramadol at 83 ng/mL; gabapentin at 5,500 ng/mL; valproic acid at 23,000 ng/mL; butalbital at 1,600 ng/mL; and caffeine. Gastric fluid contained tramadol at 3.6 mcg/mL, gabapentin at 56 mcg/mL, and fluoxetine at 3.4 mcg/mL, with qualitative detections of acetaminophen, caffeine, nicotine, and beta-phenethylamine. Substantial gastric fluid drug concentrations are consistent with recent ingestion before death.

Discussion: Findings are consistent with fatal mixed-drug intoxication involving methadone and multiple co-intoxicants with overlapping central nervous system depressant effects. A major limitation is the absence of peripheral blood, the preferred specimen for postmortem interpretation; cardiac blood concentrations may be elevated by postmortem redistribution and should be interpreted accordingly. The methadone concentration falls within a range that may overlap with therapeutic or maintenance levels; however, prescription status was unconfirmed, and direct comparison of cardiac blood concentrations with published reference ranges derived largely from peripheral blood is not considered best practice in postmortem forensic toxicology. Co-detection of tramadol, 7-aminoclonazepam, gabapentin, butalbital, and

valproic acid may have contributed to sedation, impaired consciousness, and respiratory compromise. Fluoxetine, a potent CYP2D6 inhibitor, may have altered tramadol metabolism by increasing tramadol concentrations and decreasing the formation of its active metabolite, O-desmethyltramadol, increasing the risk of seizures and serotonin syndrome. Fluoxetine and norfluoxetine blood concentrations, along with fluoxetine detected in gastric fluid, indicate significant antidepressant exposure; however, fluoxetine undergoes substantial postmortem redistribution (reported cardiac/peripheral blood ratio 2.83), and the absence of peripheral blood limits interpretation. Prior suicide attempts and recurrent overmedication were noted, although the manner of death was certified as accident. This case highlights the importance of integrating investigative history, prescription records, tolerance assessment, cardiac and peripheral blood findings, and gastric fluid results when evaluating deaths involving polypharmacy and multiple central nervous system depressants.

Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

P-345

Withdrawn

Withdrawn

P-346

Fatal Polydrug Intoxication in a Chemsex Context: Forensic Challenges in the Interpretation of Synthetic Cathinones

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Abstract

Introduction: Chemsex involves the use of psychoactive substances to facilitate, enhance or prolong sexual experiences. Commonly involved drugs include methamphetamine, GHB/GBL, ketamine, alkyl nitrites ("poppers"), MDMA, cocaine, cannabis and, increasingly, new psychoactive substances (NPS) such as synthetic cathinones.

We report a fatal case of polysubstance intoxication in a chemsex setting, involving two synthetic cathinones (MDPHP and α-PVP) and other drugs. The case concerned a 28-year-old male found deceased after a chemsex encounter with reported polysubstance use. Synthetic cathinones are potent psychostimulants, and α-pyrrolidinophenone derivatives, a subclass of cathinones, are characterized by high potency, prolonged effects, and limited post-mortem data.

Objectives: This study describes a fatal case of polydrug use related to chemsex involving two synthetic cathinones. The objectives are to highlight the toxicological findings, examine interpretative challenges and emphasize forensic implications. This case seeks to contribute to the limited post-mortem data on synthetic cathinones and to raise awareness of their role in chemsex-related deaths.

Methods: Postmortem toxicological analysis was performed on peripheral blood, urine, gastric content and vitreous humor. Initial screening for common drugs of abuse was carried out by immunoassay techniques, followed by confirmatory analysis by gas chromatography-tandem mass spectrometry (GC-MS/MS).

Prior to instrumental analysis, biological samples were subjected to matrix-dependent extraction procedures. Liquid–liquid extraction (LLE) was applied to the urine sample, while solid-phase extraction (SPE) was used for blood, gastric content and vitreous humor.

Then, targeted and expanded screening for drugs and new psychoactive substances was subsequently performed using liquid chromatography–tandem mass spectrometry (LC–MS/MS) and ultra-high-performance liquid chromatography-high-resolution mass spectrometry (UHPLC-Q Exactive Orbitrap).

Results: Toxicological analysis revealed extensive polydrug use. Two synthetic α-pyrrolidinophenone cathinones, MDPHP and α-PVP, were detected in blood and urine. Additionally, methamphetamine, cocaine, morphine and benzodiazepines were also identified in all matrices. Gastric content analysis supported recent drug intake.

Methamphetamine was detected in peripheral blood at concentrations consistent with toxic effects. Although MDPHP and α-PVP were confirmed, quantification was limited by their chemical instability and the potential post-mortem degradation, particularly as autopsy was performed 24 hours after death.

Discussion: This work illustrates the toxicological challenges of polydrug chemsex-related fatalities. Interpretation of post-mortem concentrations is further complicated by tolerance, binge use patterns and potential post-mortem redistribution. While the cause of death remains undetermined, the findings are consistent with an acute adverse drug reaction.

Despite quantitative limitations, the presence of two α-pyrrolidinophenone synthetic cathinones suggests a significant contribution to stimulant toxicity. The concomitant presence of methamphetamine, cocaine, opioids and benzodiazepines increased the risk of adverse cardiovascular and central nervous system effects.

Overall, this case highlights the need to continuously update analytical strategies and generate post-mortem data in chemsex settings to improve forensic interpretation of synthetic cathinones and other emerging substances.

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Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

P-347

LC-MS/MS Chiral Analysis of Ketamine in Danish Drug Seizures and DUID Blood Samples: Forensic Implications of Racemic Prevalence

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Abstract

Introduction: Ketamine is used medically as an anesthetic and antidepressant drug, but is also used recreationally for its dissociative, sedative, and hallucinogenic effects. In Denmark only *S*-ketamine is marketed for therapeutic purposes. The enantiomeric composition of ketamine found in seized drugs and ketamine positive blood samples from Driving Under the Influence of Drugs (DUID) cases in Denmark has not yet been investigated.

Objectives:

1. To investigate whether *S*- or racemic ketamine is present in seized drug samples.
2. To investigate the occurrence of *R*- and *S*-ketamine in blood samples from DUID cases.

Methods: Chiral separation and quantification of *R*/*S*-ketamine were performed using LC-MS/MS with a CHIRALPAK AGP column (2.1mmIDx100mm, 5µm particle size, Daicel Corporation) coupled to a Xevo TQ-S micro (Waters). The mobile phases consisted of 10 mM ammonium formate and 2-propanol. Ketamine-D3 was used as internal standard.

Sample preparation of seized drugs was done by dissolving 50 mg of the sample in 5 ml solvent mixture (acetonitrile/methanol/H_2O, 25:25:50) followed by a 1000-fold dilution with the same solvent.

Blood sample preparation involved 200 µl of blood, which was protein-precipitated with 800 µl acetonitrile. The supernatant was centrifuged through a 30kDa filter; 500 µl of the filtrate was evaporated to dryness and reconstituted in 200 µl of 15% methanol in water.

Results: The chromatographic separation of *R*/*S*-ketamine peaks achieved a resolution of R_s = 1.19. Comparing the sum of *R*- + *S*- ketamine concentration to our validated (ISO17025) achiral ketamine method revealed an average difference of 3% for blood samples and 9% for drug seizures. All the drug seizures (n=32) were racemic ketamine. These samples were typically white powders with a purity range of 26—83%. Analyzing DUID blood samples (n=72) revealed that 40% contained pure *S*-ketamine and 60% contained racemic ketamine, with a median (min—max) concentration of 0.19 (0.031—0.97) and 0.016 (0.0006—0.89) mg/kg, respectively. The ratio of *R*- to *S*-ketamine in the racemic samples had a median (min—max) of 1.02 (0.85—1.4) with a slight tendency toward to higher ratios at lower total ketamine concentrations.

Discussion: Only racemic ketamine was found in the investigated seized drugs, this was expected, as *S*-ketamine is significantly more expensive to produce. The identification of racemic ketamine in blood samples indicates illicit use, since all medicinal ketamine in Denmark is *S*-ketamine. Interpreting *S*-ketamine findings is challenging because, while used clinically, it may also reflect the consumption of non-prescribed medication or the availability of *S*-ketamine on the illicit market. The 60% prevalence of racemic ketamine in DUID samples suggests that enantioselective monitoring is a valuable tool for characterizing ketamine use patterns.

The median ketamine concentration found for *S*-ketamine (0.19mg/kg) was higher than racemic ketamine (0.016mg/kg). We speculate that this is because blood sampling is closer to intake in therapeutic use (e.g., treating trauma) compared with DUID cases, where drug intake may have occurred several hours prior. The tendency of higher *R*/*S*-ketamine ratios at lower total concentrations could indicate stereoselective metabolism with a higher affinity for the *S*-enantiomer.

Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

P-348

Oral Fluid Versus Blood for Delta-9-Tetrahydrocannabinol and Cocaine: Concordance, Discordance, and Limits of Concentration Prediction

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Abstract

Introduction: Oral fluid is increasingly used in forensic settings because collection is simple, non-invasive, and operationally attractive. However, the interpretation of oral-fluid results remains a matter of debate, particularly when these results are compared with blood concentrations.

Objectives: We assessed the relationships between oral-fluid and blood findings for delta 9-tetrahydrocannabinol (THC) and cocaine, focusing on qualitative concordance, quantitative association, and the ability of oral fluid to predict blood concentrations.

Methods: In the context of roadside drug testing, paired oral-fluid and blood results obtained from the same subjects were retrospectively evaluated. Oral-fluid specimens were collected using FLOQSwabs® (Copan, Apri 2000, France). For cannabis exposure, THC was considered, yielding 72 paired samples. For cocaine exposure, 46 paired samples were available. Cocaine-related analytes considered in interpretation were cocaine, benzoylecgonine (BZE), and ecgonine methyl ester (EME). Two interpretive frameworks were applied for cocaine: a strict parent-compound approach and an exposure-oriented approach in which detection of a cocaine-related metabolite was considered evidence of cocaine use and therefore not discordant with an oral-fluid-positive result. Qualitative concordance/discordance rates were calculated, and quantitative relationships were explored in pairs positive in both matrices. The potential impact of blood storage delay before analysis was also assessed.

Results: For THC, 29/72 cases (40.3%) were positive in both matrices, 21/72 (29.2%) were negative in both, and 22/72 (30.6%) were oral-fluid-positive/blood-negative; no blood-positive/oral-fluid-negative case was observed. Overall qualitative concordance was 69.4%, but all discordance was unidirectional, with oral fluid detecting THC in the absence of blood detection. Among the 29 pairs quantifiable in both matrices, only a moderate association was observed between oral-fluid and blood concentrations (Pearson $r = 0.54$; Spearman rho = 0.53). The oral-fluid-to-blood ratio was highly variable and increased with oral-fluid concentration, ranging from 0.75 to 720 (Pearson $r = 0.803$), indicating that no simple conversion factor between oral fluid and blood could be supported. Blood storage delay had no detectable impact on the observed oral-fluid/blood relationships. For cocaine, under a strict parent-compound interpretation, only 8/46 cases (17.4%) were concordant: 4 oral-fluid-positive/blood-positive and 4 oral-fluid-negative/blood-negative. When blood samples positive for cocaine-related metabolites were considered evidence of cocaine exposure, concordance increased to 17/46 cases (37.0%). Nevertheless, 29/46 cases (63.0%) remained discordant, all corresponding to oral-fluid-positive/blood-negative results without blood evidence of cocaine or related metabolites. No oral-fluid-negative case with blood evidence of cocaine exposure was observed. Blood storage delay likewise showed no meaningful influence.

Discussion: For both THC and cocaine, swab-collected oral fluid appeared to be a sensitive

matrix for documenting recent exposure, but not a reliable surrogate for blood. For THC, the moderate correlation and extreme variability of the oral-fluid-to-blood ratio precluded quantitative prediction of blood concentration from oral fluid. For cocaine, even when cocaine-related metabolites were incorporated into interpretation, concordance remained limited and quantitative extrapolation was clearly untenable. Overall, for the swab-based sampling device used here, oral fluid should be regarded as a matrix of exposure detection rather than a matrix for reconstructing blood concentrations.

Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

P-349

Prevalence of Alcohol Among Drivers, Riders, and Pedestrians Injured in Road Traffic Crashes in Cameroon: A Cross-Sectional Study

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Abstract

Introduction: Road traffic crashes are a major public health issue in Cameroon, with alcohol consumption identified as a significant contributing factor. Reliable data on alcohol use among injured road users is limited, particularly in low- and middle-income countries.

Objectives: This study aimed to determine the prevalence of alcohol among injured drivers, motorcycle riders, and pedestrians admitted to three major hospitals in Cameroon, and to examine associations with socio-demographic and injury characteristics.

Methods: A cross-sectional study was conducted among 350 injured road users admitted to hospitals in Douala and Yaoundé. Alcohol use was measured using breathalyzers, and data on age, gender, education, religion, type of road user, crash details, and injury severity were collected via questionnaire. Multivariable logistic regression was applied to assess associations.

Results: Overall, 30.9% of participants had blood alcohol concentrations (BACs) above 0.08% (legal limit for drivers). The highest prevalence was observed among motorcycle riders (36.5%), followed by pedestrians (24.8%) and drivers (18.9%). Elevated BACs were most frequent during weekend nights and among seriously injured patients. Participants identifying as Muslim had significantly lower prevalence of alcohol use.

Discussion: Alcohol consumption is a major contributor to road traffic injuries in Cameroon, particularly among motorcycle riders. Many injuries could have been prevented in the absence of alcohol use. Targeted interventions, including stricter enforcement and awareness campaigns, are needed to reduce alcohol-impaired road use and its associated burden.

Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

P-350

Drug-Facilitated Sexual Assault in Israel: A Retrospective Toxicological Study (2020–2025) Examining Post–Policy Change Trends

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Abstract

Introduction: Drug-facilitated sexual assault (DFSA) is a growing concern in Israel and is often underreported due to delayed victim awareness, drug-induced memory impairment, and limited specialized forensic responses. These challenges hinder effective investigation, reduce substance detection rates, and contribute to low prosecution and conviction rates. In response, the Israeli Ministry of Health implemented a policy in December 2018 mandating standardized toxicological screening in suspected DFSA cases.

Objectives: To evaluate the impact of the national policy on case submissions and detection, and to describe demographics, sampling timelines, and toxicological findings from 981 suspected DFSA cases tested at the Clinical Toxicology and Pharmacology Laboratory (CTPL) in Sheba Medical Centre between 2020 and 2025.

Methods: Urine and/or blood samples from alleged victims were collected and analyzed at the CPTL. Ethanol was detected using headspace gas chromatography, while Cannabis, Cocaine, Benzodiazepines, Amphetamines and Opiates were screened using enzyme immunoassays. Analytical cutoffs were according to the Federal Registers guidelines. Confirmatory analyses were performed using gas chromatography–mass spectrometry (GC-MS) and high-performance liquid chromatography-mass spectrometry (LC-MS-MS). Data on sample collection timing, victim demographics, and detected substances were compiled and analyzed over the six-year study period.

Results: Case submission rose nearly ninefold, from 27 in 2020 to 236 in 2025, indicating improved detection and reporting following the policy. The median victim age remained 21 to 25 years, and ~90% were female. 46% of the victims presented within 12 hours of the alleged assault. Overall, 28% of cases yielded positive toxicology, with a substantial concentration of positives among those sampled within 12 hours, underscoring the time sensitivity of detection.

Substance detection revealed ethanol as the most prevalent (range 21-51%), followed by cannabis (8-33%), cocaine (8-15%), MDMA (4-6%), ketamine (up to 13%), and Methamphetamine (up to 18%). There was A notable rise in ketamine in 2024 and Methamphetamine in 2025. Frequently detected non-illicit medications included analgesics (6–18%), antidepressants (5–16%), benzodiazepines (2–13%), and antipsychotics (3–9%).

Discussion: Before 2018, testing was decentralized and inconsistent across hospitals, limiting comparability; after policy implementation, early adoption was modest, with robust, standardized data available from 2020 onward. Nationwide training programs for healthcare personnel focused on medico-legal procedures, and a network of 11 specialized acute treatment centers for sexual assault, established since 2000, enhanced victim support by confirming substance use, improving

medical and psychological care, and gathering data on substance trends to inform prevention and policy, ensuring professionals were better equipped to manage DFSA cases and improving victim outcomes.

Analyses were restricted to hospital-admitted cases tested within 96 hours, potentially underestimating late-presenting DFSA. The testing panel did not cover some potential DFSA agents (e.g., volatile anesthetics, certain novel/designer drugs). Limited self-reported drug histories and long drug/metabolite half-lives constrained differentiation between voluntary and involuntary use.

In DFSA cases, standardized, mandatory toxicology and prompt sampling, ideally within 12 hours, significantly increase substance detection, strengthen forensic detection and documentation, and support legal proceedings and victim care. Sustained surveillance, ongoing professional training, and expanded analytical panels are also critical to further improve detection, victim support, and judicial outcomes.

Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

P-351

Establishment of a Medication Compliance Threshold for Probationers Ordered to Take Quetiapine, Risperidone, and Aripiprazole Using Urinalysis and ROC (Receiver Operating Characteristics) Analysis

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Abstract

Introduction: In Korea, a probation system is in effect that imposes the use of antipsychotic drugs on offenders with mental illnesses based on court orders or requests from the prosecution. Since 2019, the Forensic Chemistry Laboratory of the Supreme Prosecutors' Office has been providing assessments of drug compliance in consultation with probation offices. However, the Forensic Chemistry Laboratory of the Supreme Prosecutors' Office has been conducting these assessments without clear threshold for determining medication compliance. This study aims to ensure clarity in assessments by establishing compliance threshold for antipsychotic medication prescribed to probationers.

Objectives: This study was conducted to establish compliance threshold for antipsychotic medication of the three most commonly prescribed antipsychotic drugs (Quetiapine (QTP), risperidone (RPD), and aripiprazole (ARPZ)) to probationers in Korea using urine.

Methods: Quantitative analyses of QTP, RPD, ARPZ, norquetiapine (NQTP), paliperidone (PPD), and dehydroaripiprazole (DHARPZ) were performed using UPLC-MS/MS on 454 urine samples (199 QTP, 121 RPD, and 134 ARPZ) submitted from the Capital Region Probation Office of South Korea to the Supreme Prosecutors' Office. Urinary creatinine concentration was measured using Cobas C311 (Roche). The quantitative analysed results were divided into 4 compliance indices(Urine Drug Concentration (UDC), Daily Dose Adjustment (DPA), Creatinine Adjustment (UDC/Cr), and DPA/Cr) considering daily drug dosage and creatinine concentration. These compliance indices underwent log transformation and standardization processes. To establish compliance threshold for prescription drugs under the probation order, receiver operating characteristics (ROC) analysis was conducted using standardized compliance indices and prescription drug use results reported by the Supreme Prosecutors' Office to Seoul Metropolitan Area Probation Offices over the past five years. Area under the curve (AUC) was used for the ROC discrimination evaluation.

Results: The AUC values of QTP, RPD, and ARPZ were 1.000, 0.894–0.970, and 0.892–0.943, respectively . The sensitivities of QTP, RPD, and ARPZ were 1.000, 0.713–0.925, and 0.892–0.943, respectively. The specificities of QTP, RPD, and ARPZ were 0.974–1.000, 0.900–1.000, and 0.857–0.962, respectively. The medication compliance threshold of each compliance indicator were calculated as follows: When using UDC (ng/mL), the medication compliance threshold of QTP/NQTP, RPD/PPD, and ARPZ/DHARPZ are 0.11/3.12, 2.62/121.49, and 10.55/1.07, respectively. When using DPA (ng/mL/mg), the medication compliance threshold of QTP/NQTP, RPD/PPD, and ARPZ/DHARPZ are 0.004/0.12, 12.00/83.86, and 0.19/0.24, respectively. When using UDC/Cr (ng/mg), the medication compliance threshold of QTP/NQTP, RPD/PPD, and ARPZ/DHARPZ are 1.59/7.49, 132.10/233.28, and 0.44/0.93, respectively. When using DPA/Cr (ng/mg), the medication compliance threshold of QTP/NQTP, RPD/PPD, and ARPZ/DHARPZ are 0.002/0.26, 2.53/63.62, and 0.23/0.14, respectively.

Discussion: The medication compliance threshold of the three antipsychotic drugs (QTP, RPD, ARPZ) presented in this study could contribute to objectively evaluating medication compliance among probationers and would be expected to be used as a practical standard for verifying medication compliance among probationers in the future.

Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

Conflict of Interest

Grant/Research

P-352

The Advantages of Fold Changes in Comparing THC Concentrations in Breath After Cannabis Use

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Abstract

Introduction: Currently there is no reliable measurement to determine if someone has recently used Δ^9-tetrahydrocannabinol (THC), the main psychoactive component of cannabis. Breath sampling is non-invasive and THC has been routinely detected in breath after cannabis use, but the large range of reported THC concentrations makes it difficult to draw further conclusions from these data. Some variability in THC is to be expected given that multiple sampling devices and different sampling protocols have been employed. Here, we consider data from 10 studies spanning three collection devices and differing sampling protocols (e.g., collection time vs. total volume). Fold change can be used to compare THC concentrations at two timepoints, showing order-of-magnitude changes without being visually biased by absolute change.

Objectives: The objective of this study is to explore THC concentration changes after cannabis use through the calculation of fold change, enabling us to compile data from ten studies despite differences in sampling devices and protocols.

Methods: Fold changes ($FC_{T1\text{-}T2}$) were calculated for subsequent post-use timepoints (T_1 and T_2). We considered T_1 data within 0.5 h ≤ T_1 ≤ 1 h, T_2 data ≤ 2 h, and time differences between T_1 and T_2 (ΔT) of 0.5 h ≤ ΔT ≤ 1 h whenever possible. When multiple timepoints were available, fold changes were calculated for each appropriate timepoint. $FC_{T1\text{-}T2}$ is calculated as $[THC]_{T2}/[THC]_{T1}$. Since the T_2 sample is collected at least 30 min after the T1 sample, $FC_{T1\text{-}T2}$ is expected to indicate a THC concentration decrease. We estimated that fold changes with a factor-of-two change (doubling or halving the THC concentration between data points) can be considered substantial for discussion purposes only.

Results: THC concentrations at T_1 span more than 5 orders of magnitude across ten studies, with 316 comparisons available to calculate fold change. $FC_{T2\text{-}T1}$ also spans five orders of magnitude. After cannabis use, 62% of $FC_{T1\text{-}T2}$ values from the ten studies show substantial decreases in THC concentration between two post-use timepoints. Based on our estimations of what could be considered substantial fold changes, 33% of $FC_{T1\text{-}T2}$ values are classified as unsubstantial changes in THC concentration. A small number of participants (5% of comparisons) had $FC_{T1\text{-}T2}$ values greater than 2, meaning those participants had an increase in THC concentration between the two post-use timepoints.

Discussion: As study designs, breath sampling protocols, and breath collection devices varied for all studies, some variability in THC concentrations can be expected; however, the span of THC concentrations at T_1 makes it challenging to compare findings. While both THC concentrations at T_1 and $FC_{T2\text{-}T1}$ span five orders of magnitude, more information can be gained from fold changes across multiple breath timepoints than from a single post-use measurement. THC concentration from T_1 to T_2 decreased by a factor of two in 62% of the total comparisons between all studies. These results indicate that fold change may be useful for combining results across studies to develop a meaningful way to determine recent use without requiring conformity between protocols and devices.

Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

P-353

Accelerating Drug Screening in Forensic Hair Analysis by Applying High-Speed Polarity Switching in HR LC-MS/MS

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Abstract

Introduction: In 2023, the Society of Hair Testing (SoHT) issued revised international guidelines for hair analysis of drugs of abuse, encompassing eight major analyte classes: opiates, cocaine, amphetamines, cannabinoids, opioids, methadone, buprenorphine, and ketamine. The updated consensus identified the increased challenge presented by new psychoactive substances (NPS) and the escalating misuse of prescription medications.

Objectives: In this study, a high-resolution QTOF LC-MS/MS workflow incorporating rapid polarity switching and data-dependent MS/MS acquisition to support comprehensive targeted screening was developed to support targeted and non-targeted workflows. This integrated approach enables high-confidence targeted and non-targeted detection of classical drugs of abuse, NPS, and prescription pharmaceuticals in forensic hair matrices.

Methods: Hair samples (each donor providing a single 6 cm sample) were segmented into six 1 cm samples to identify drug use over an estimated 6-month timeframe. Samples were measured by high resolution LC-MS/MS analysis (QTOF LCMS-9050, Shimadzu Corporation). The method acquired a TOF-MS scan (m/z 100-1000; 100 msec) followed by DDA-MS/MS scans (m/z 40-1000; 33 msec, 4 dependent events) in positive ion mode followed by a TOF-MS and DDA-MS/MS scans in negative ion mode (same mass range and scan time, 2 dependent events) resulting in a total cycle of less than 2 seconds. LabSolutions Insight Profiler was used for feature detection, data alignment and automated compound identification.

Results: In one subject, targeted screening identified cocaine and related metabolites, norcocaine, benzoylecgonine, anhydroecgonine methyl ester, and cocaethylene with the highest concentrations detected in distal hair segments (5–6 cm) compared to proximal segments (0–1 cm), indicating reduced consumption over time. Quantitative analysis confirmed cocaine at 1.24 ng/mg in the 5–6 cm segment, decreasing to <0.01 ng/mg in the 0–1 cm segment, well below the 0.5 ng/mg reporting cutoff.

In another subject, targeted screening revealed high levels of aripiprazole and risperidone (antipsychotics), trazodone (antidepressant), m-chlorophenylpiperazine (m-CPP psychoactive), cocaine, codeine, and associated metabolites. Given that trazodone is metabolized to m-CPP primarily via CYP3A4, the elevated trazodone-to-m-CPP ratio suggests *in vivo* metabolism rather than direct m-CPP ingestion.

Discussion: A high-resolution QTOF LC-MS/MS method was developed to acquire full-scan TOF-MS data with data-dependent MS/MS (DDA) acquisition and rapid polarity switching to deliver a targeted screening of drugs of abuse, new psychoactive substances (NPS) and prescription medications in hair samples. To accelerate data processing and automate high confidence reporting,

a novel data-processing application, Insight Profiler, was developed. The software automates feature detection, alignment, statistical filtering, and compound identification within a single processing method. The approach supports both targeted workflows, using MS/MS spectral library matching with verified retention times, and non-targeted workflows, leveraging third-party MS/MS databases without retention time constraints.

The Insight Profiler method executes a cascade of automated steps for both workflows, including advanced feature detection, inter-sample feature alignment, statistical filtering, and compound identification using curated MS/MS libraries or suspect screening lists. Application of this methodology revealed several natural products and over-the-counter (OTC) drugs, such as paracetamol and the antihistamine cetirizine.

Disclosure

Yes, I, or a member of my immediate family, has a financial interest to disclose.

P-354

Passive Cocaine Exposure Through Kissing or Sexual Contact

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Abstract

Introduction: The detection of cocaine and its metabolites in biological samples is generally interpreted as a direct indication of drug use. However, the possibility of passive transfer via kissing or sexual intercourse remains a contentious issue in forensic toxicological assessments.

Objectives: The aim of this study was to evaluate the forensic toxicological findings following the detection of cocaine and its metabolites in a case where the individual claimed not to have used illicit substances, and to discuss the possibility of passive transfer.

Methods: Based on intelligence information, a forensic search was conducted during which ecstasy (MDMA) tablets and cannabis (THC) were seized at the scene. Blood and urine samples were collected from a woman and a man who were present at the scene and were reported to be in a romantic relationship. However, there was an 18-day interval between the incident and the collection of the biological samples. The blood and urine specimens were analyzed at the Toxicology Department of the Adana Council of Forensic Medicine using liquid chromatography–tandem mass spectrometry (LC–MS/MS). Subsequently, following the woman's objection, the court ordered hair segment analysis to evaluate her history of drug use. Hair samples were collected from the woman approximately six months after the incident and were analyzed by LC–MS/MS at the Department of Forensic Medicine, Cukurova University. For urine analysis, the LODs/LOQs were 0.37/1.11 ng/mL for cocaine and 0.27/1.00 ng/mL for benzoylecgonine. For hair analysis, the LODs/LOQs were 5/15 pg/mg for cocaine and 1/5 pg/mg for benzoylecgonine.

Results: The toxicological analysis of the male subject's blood sample revealed the presence of THC-COOH (2.85 ng/mL), cocaine (1 ng/mL), benzoylecgonine (41.35 ng/mL), and ecgonine methyl ester (1.15 ng/mL). Urine analysis from the same individual was positive for MDA, MDMA, cocaine, benzoylecgonine, ecgonine methyl ester, and THC-COOH. In the female subject's blood sample, benzoylecgonine was detected at a concentration of 1 ng/mL, while her urine sample tested positive for cocaine and benzoylecgonine. The female subject's 30-cm hair sample was sectioned into eight consecutive segments from the proximal (root) to the distal end and analyzed separately. No illicit substances were detected in any of the hair segments. Considering the negative findings of the segmental hair analysis, the court acquitted the female defendant.

Discussion: Passive cocaine exposure through kissing or sexual intercourse has been documented in the literature, whereby cocaine metabolites — particularly benzoylecgonine — may be detected in biological fluids of non-using individuals following intimate contact with an active user. Crucially, segmental hair analysis serves as a reliable retrospective marker of chronic drug exposure, as each centimetre of hair approximately reflects one month of growth; the absence of any illicit substances across all eight segments of the 30 cm hair sample effectively excluded habitual or long-term cocaine use by the female subject. The court's decision to acquit the female defendant underscores the indispensable role of segmental

hair analysis in forensic toxicology, particularly in cases where passive exposure must be distinguished from active drug use.

Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

P-355

Detection of Fentanyl and Analogs in Oral Fluid by Homogeneous Enzyme Immunoassay

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Abstract

Introduction: Fentanyl is a highly potent synthetic opioid and Schedule II substance that binds to the μ-opioid receptors. It is highly abused and continues to contribute significantly to the opioid crisis in the United States. The increasing number of novel synthetic analogs (commonly referred to as *fentalogs*) that are illegal and were classified as Schedule I substances in 2025, present significant analytical challenges for forensic toxicology laboratories. While urine remains the most commonly used testing matrix, oral fluid offers several advantages, including observed collection, reduced adulteration risk, and preferential detection of parent compounds. Oral fluid specimens collected using the Quantisal™ device provide volume-controlled sampling and standardized dilution of 1 mL of specimen with 3 mL of stabilization buffer, making this matrix suitable for fentanyl screening.

Objectives: To develop a sensitive homogeneous enzyme immunoassay (HEIA™) for detection of fentanyl and structurally related analogs for screening applications, that meets the SAMHSA recommended fentanyl cutoff of 4 ng/mL in neat oral fluid.

Methods: The assay employs a polyclonal antibody designed with broad cross-reactivity to fentanyl analogs and a matched fentanyl labeled-glucose-6-phosphate dehydrogenase enzyme conjugate. Fentanyl calibrators (0, 2, 4, 8, 20 ng/mL) and controls (3 and 5 ng/mL) were prepared in synthetic oral fluid and diluted 1:4 with oral fluid extraction buffer, to simulate the Quantisal™ sample processing. Testing was performed on a Beckman Coulter AU5800 chemistry analyzer using a 15 µL sample volume, 90 µL and 45 µL RA/RE volumes respectively and absorbance was measured at 340 nm. Both qualitative and semi-quantitative analyses were evaluated. Imprecision studies, as well as cross-reactivity studies using structurally related opioids and non-related compounds (fortified at 40,000 ng/mL in neat oral fluid), endogenous substances and commonly ingested substances (fortified at ± 25% cutoff) were performed.

Results: The HEIA™ successfully detected fentanyl at a 4 ng/mL cutoff in neat oral fluid. Inter-day imprecision (n=80), using semi-quantitative analysis was ≤10%, while qualitative analysis showed ≤4% variability. The assay demonstrated broad cross-reactivity with numerous fentanyl analogs, including acetyl fentanyl (100%), 4-fluoro-isobutyryl fentanyl (89%), furanyl fentanyl (80%), acryl fentanyl (80%), valeryl fentanyl (80%), p-fluorofentanyl (80%), isobutyryl fentanyl (73%), cyclopropyl fentanyl (67%), octfentanil (67%), methoxyacetyl fentanyl (67%), cyclopentyl fentanyl (67%), p-fluorobutyryl fentanyl (67%), butyryl fentanyl (67%), (±)-cis-3-methylfentanyl (53%), p-chloroisobutyryl fentanyl (53%), p-methoxybutyryl fentanyl (50%), β-hydroxy fentanyl (44%) and (±)-β-hydroxythiofentanyl (40%). No cross-reactivity was observed with non-related compounds or common interfering substances. Method comparison using 203 oral fluid specimens showed 96 true positives and 105 true negatives, with only one false positive (3.75 ng/mL) and one false negative (5.75 ng/mL), both near the cutoff, yielding an overall agreement of 99% compared to LC-MS/MS.

Discussion: A highly sensitive HEIA™ for fentanyl and numerous analogs has been developed that meets the SAMHSA-recommended screening cutoff of 4 ng/mL for fentanyl in oral fluid. This assay supports the use of oral fluid as a reliable alternative matrix to urine for forensic toxicology screening of fentanyl and fentanyl analog misuse.

Disclosure

Yes, I, or a member of my immediate family, has a financial interest to disclose.

Conflict of Interest

All authors receive salary from Abbott Rapid Diagnostics

P-356

Concentration Distributions and Metabolite-to-Parent Ratios in Hair Toxicology: A Retrospective Study

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Abstract

Introduction: Hair toxicology offers a unique analytical advantage by providing a prolonged detection window for drug exposure, enabling retrospective exposure assessment that is particularly valuable in clinical, forensic, and child-protection contexts. In recent years, increasing concern has focused on unintentional and environmental drug exposures, especially to fentanyl and stimulants, among pediatric populations. Interpretation of hair results, however, remains complex and may benefit from evaluation of metabolite detection patterns and metabolite-to-parent drug ratios to better distinguish systemic exposure from external contamination.

Objectives: The objective of this study was to characterize concentration distributions and metabolite-to-parent ratios in hair specimens submitted for clinical and forensic testing, with particular emphasis on fentanyl, methamphetamine/amphetamine, and cocaine. Secondary objectives included evaluation of demographic characteristics, submission context, and the interpretive value of metabolite ratios.

Methods: A retrospective analysis was conducted using hair toxicology data collected between January 2024 and December 2025. Hair samples were analyzed using validated quantitative LC–MS/MS methods; quantitative analytical data were leveraged for research purposes, although hair drug results are currently reported qualitatively. Samples were decontaminated with methylene chloride, followed by drying, grinding, and methanolic incubation for two hours at 45 °C. Analytes evaluated included fentanyl, norfentanyl, and 4-ANPP; methamphetamine and amphetamine; cocaine and benzoylecgonine (BZE). Concentration ranges, means, and medians (ng/mg) and metabolite-parent ratios were calculated. Available demographic information and submission type were also assessed.

Results: The dataset comprised 36 fentanyl-positives, 101 methamphetamine-positives, and 49 cocaine-positives cases, all from full-length hair samples. A substantial proportion of positive findings occurred in pediatric populations, particularly young children and school-aged individuals. Clinical reference laboratory or hospital submission predominated across all cases.

Reported concentrations demonstrated substantial variability. Fentanyl concentrations ranged from 0.0025–3.76 ng/mg (reporting limit [RL] 0.002; mean 0.64±0.95; median 0.075). Norfentanyl and 4-ANPP were frequently detected at lower concentrations, with median NOR/FEN and ANPP/FEN ratios of approximately 0.02 and 0.13, respectively. Methamphetamine concentrations ranged from 0.024–65.6 ng/mg (RL 0.010; mean 4.21±8.53; median 1.23), with low relative amphetamine concentrations (median AMP/MAMP 0.04). Cocaine concentrations ranged from 0.19–388 ng/mg (RL 0.19; mean 24.3±63.0; median 1.47) with median BZE/COC ratios of 0.24.

Metabolite detection patterns varied by analyte. Amphetamine was detected in 98% of methamphetamine-positive, compared with benzoylecgonine in 59% and norfentanyl in 50% of cocaine- and fentanyl-positive cases. Of note, 4-ANPP—both a minor fentanyl metabolite and a

known precursor material in illicit fentanyl production processes—was detected in 90% of fentanyl-positive cases. Predominantly pediatric cases (61-75%) limited age-correlation assessment; therefore, age-related effects were not evaluated.

Discussion: These findings demonstrate frequent detection of fentanyl and stimulants in hair across both clinical and forensic submissions, with notable pediatric representation. Evaluation of concentration distributions in conjunction with metabolite-to-parent ratios provides additional interpretive value beyond qualitative detection alone. Although not independently diagnostic, drug-metabolite ratios provide enhanced interpretive context, particularly when distinguishing systemic exposure from environmental contamination. These data support continued research into quantitative hair toxicology and ratio-based interpretation frameworks as complementary tools in clinical and forensic practice.

Disclosure

Yes, I, or a member of my immediate family, has a financial interest to disclose.

Conflict of Interest

Salary

P-357

Optimised Sample Preparation for Drugs of Abuse Extraction From Oral Fluid Prior to UHPLC-MS/MS Analysis

Lucy Richards, Russell Parry, Helen Lodder, Charlotte Hayes, Lee Williams, Adam Senior, Zainab Khan, Geoff Davies, Claire Desbrow

Biotage, Cardiff, Cardiff, United Kingdom

Abstract

Introduction: Recent regulatory changes have resulted in oral fluid becoming the preferred matrix for roadside and forensic drug testing due to its tamper-proof and non-invasive sample collection. Despite these advantages, the analysis of oral fluid comes with its challenges, particularly due to the presence of surfactants, salts and preservatives within the collection buffer. These components can adversely affect analyte recovery and chromatographic performance, leading to matrix effects and reduced method sensitivity. An efficient sample preparation method is therefore essential to mitigate buffer related interferences and ensure reproducibility and robustness prior to analysis.

Objectives: This poster will present optimized sample preparation methodology for the extraction of commonly encountered drugs of abuse from oral fluid using mixed-mode strong cation exchange SPE.

Methods: A typical drugs of abuse analyte panel was spiked at various concentrations ranging from 0.1 to 100 ng/mL and extracted from oral fluid collected using the Quantisal collection devices. Sample extraction was performed using polymer-based mixed-mode strong cation exchange solid phase extraction, EVOLUTE EXPRESS CX. Key extraction parameters were evaluated and optimized to maximize analyte recovery and reduce buffer derived interferences. Samples were processed using positive pressure and concentrated to dryness with a TurboVap 96 Dual evaporator. Analysis was performed using a Shimadzu Nexera UHPLC coupled to an 8060 triple quadrupole mass spectrometer. Chromatographic separation was achieved using a Restek Raptor Biphenyl column and ammonium formate and formic acid in water and MeOH mobile phases.

Results: A comprehensive drugs of abuse panel including amphetamines, opioids, benzodiazepines, fentanyls, cocaine, gabapentin and pregabalin along with other commonly analyzed drugs were extracted from oral fluid using 30 mg 96-well plate formats. Full scan experiments illustrated sub optimal extract cleanliness when using traditional SPE protocols. Method development strategies were therefore focused on maximizing extract cleanliness by eliminating background interference from the oral fluid device without compromising analyte recovery. Optimization of pH control, wash and elution solvent composition, resulted in chromatograms demonstrating minimal background interference. Removal of numerous broad, unresolved peaks from the oral fluid device avoided charging issues previously observed and reduced instrument maintenance and downtime. Matrix factors greater than 0.9 were returned by the majority of analytes, again illustrating the benefit of the clean chromatographic baseline. Spiking experiments performed at various concentration levels illustrated most analytes returning high, reproducible recoveries, typically greater than 80%. A handful of analytes demonstrated recoveries lower than expected but assay sensitivity was more than adequate to overcome this issue. Precision was also excellent, with nearly all analytes demonstrating relative standard deviations below 10%, highlighting the method's reproducibility. Calibration curves were prepared across the range of 0.1 – 100 ng/mL with all analytes demonstrating good linearity and coefficients of determination, r^2 values greater than 0.99.

Discussion: The optimized CX extraction method provides a robust and efficient workflow for drug driving testing, delivering reproducible and clean extracts that support accurate quantification of drugs of abuse from oral fluid samples. Full results, discussion and conclusions will be presented in the final poster.

Disclosure

Yes, I, or a member of my immediate family, has a financial interest to disclose.

Conflict of Interest

Employed by Biotage.

P-358

Optimised Sample Preparation for Cannabinoids Extraction From Oral Fluid Prior to UHPLC-MS/MS Analysis.

Lucy Richards[1], Russell Parry[1], Charlotte Hayes[1], Helen Lodder[1], Lee Williams[1], Geoff Davies[1], Adam Senior[1], Zainab Khan[1], Claire Desbrow[2], Esraa AboJasser[1]

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Abstract

Introduction: Recent legislative and regulatory developments in drug driving enforcement have resulted in oral fluid becoming an alternative biological matrix to blood and urine for roadside and forensic testing. This shift reflects the practical advantages of oral fluid, including its non-invasive sample collection and reduced risk of tampering. Cannabinoids are a primary focus of drug driving enforcement, highlighting the need for a robust, reliable and selective sample preparation process and analysis.

However, oral fluid comes with analytical challenges, particularly due to the presence of surfactants, preservatives and salts within the collection buffer. Consequently, efficient and reproducible sample preparation strategies are critical to ensure accurate detection and quantification of cannabinoids.

Objectives: This poster will evaluate the use of SPE methodologies for the extraction of cannabinoids from oral fluid, focusing on the optimisation of the sample preparation to enhance analyte recovery and minimise matrix effects. Sample extract cleanliness will be discussed as well as linearity, recovery, precision and matrix effects for both AX and WAX media.

Methods: A cannabinoid panel was spiked at various concentrations and extracted from oral fluid collected using Quantisal oral fluid collection devices. Sample extraction was performed using polymer-based mixed-mode anion exchange and weak anion exchange solid phase extraction, EVOLUTE EXPRESS AX and WAX. Key parameters of the sample preparation procedure were optimized to maximize analyte recovery and reduce buffer derived interferences, ultimately reducing instrument maintenance and downtime. Samples were processed under positive pressure using the Biotage PRESSURE+ 96 manifold and then evaporated to dryness with a TurboVap 96 Dual evaporator. Analysis was performed using a Shimadzu Nexera UHPLC coupled to an 8060 triple quadrupole mass spectrometer. Chromatographic separation was achieved using an ACE C18 column and acetic acid in water and methanol mobile phases.

Results: The cannabinoid panel, consisting of THC, THC-OH, THC-COOH, CBD and CBN was extracted from oral fluid using 10 and 30 mg 96-well plate formats. Sample cleanliness was evaluated using various SPE chemistries using full scan LC/MS experiments. Surfactant removal was assessed by investigation of chromatographic baseline and interference profiles. This performance was further supported by matrix factors consistently exceeding 0.9 for optimised protocols. Spiking experiments found reproducible recoveries typically over 60% along with excellent precision, indicated by relative standard deviations below 10%. Calibration curves were prepared over a concentration range of 0.1 to 100ng/mL, with all analytes demonstrating strong linearity and coefficients of determination exceeding 0.99.

Discussion: Overall, the optimized AX and WAX extraction methods provide a reliable and efficient approach for extracting cannabinoids from oral fluid, consistently producing clean and reproducible extracts that enable accurate quantification. Full results, discussion and conclusions will be presented in the final poster.

Disclosure

Yes, I, or a member of my immediate family, has a financial interest to disclose.

Conflict of Interest

Employed by Biotage.

P-359

ALDH2 Deficiency Exacerbates APAP-Induced Liver Injury via the ALOX12–12-HETE Axis

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Abstract

Introduction: Carried by nearly 8% of the global population, the aldehyde dehydrogenase 2 (ALDH2) E487K gene mutation causes ALDH2 deficiency and serves as a risk factor for liver injury. Acetaminophen (APAP) is a widely used over-the-counter analgesic and antipyretic. Its overdose is a leading cause of drug-induced liver injury, accounting for 46% of acute liver failure cases in the United States. While APAP can modify ALDH2 post-translationally, the mechanisms by which ALDH2 deficiency aggravates APAP-induced liver injury (AILI) remain unclear. In this study, we used the metabolomics and proteomics methods to investigate the effects of ALDH2 deficiency on AILI.

Objectives: To investigate the mechanisms by which ALDH2 deficiency aggravates AILI in *ALDH2*2* knock-in and WT mice.

Methods: Wild-type (WT) and *ALDH2*2* knock-in mice received 500 mg/mL of APAP, while liver tissues were collected after 24 h. Hematoxylin & Eosin (H&E) staining was performed on liver sections to evaluate centrilobular necrosis and inflammatory infiltration. Mitochondrial morphology was observed under a JEM-1400-FLASH Transmission Electron Microscope. Serum levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST), malondialdehyde (MDA), and lactate were measured by commercial immunoassay kits. Using a Liquid Chromatography (LC)-Quadrupole-Orbitrap Mass Spectrometer (MS) and an LC-Quadrupole/Trap MS on liver tissue extracts, the assessment of untargeted metabolomics profile and the targeted analysis of oxidized lipids were performed, respectively. Using data-dependent acquisition (DDA) mode, fragmentation patterns were identified against an in-house and public databases, including Mass Bank, lipidMaps, mzCloud, and HMDB. For proteomics, LC combining an Orbitrap Exploris 480 MS system in data-independent acquisition (DIA) mode were applied to liver tissue, with the Wukong Cloud Platform for protein identification and quantification. Specific protein levels in proteomics were verified by Western Blot.

Results: Following APAP administration, *ALDH2*2* mice showed significantly increased central lobular degeneration and necrosis, abnormal mitochondrial morphology, elevated ALT, AST, MDA, and lactate levels compared to WT mice in the liver. Oxidative stress was exacerbated, with reduced glutathione (GSH) in metabolomics. Taregeted analysis of oxidized lipids revealed a marked increase in 12-hydroxyeicosatetraenoic acid (12-HETE) in the arachidonic acid metabolism pathway. Proteomics further identified upregulation of arachidonate 12-lipoxygenase (ALOX12), positively correlated with 12-HETE levels, which were further verified by Western blot. These findings suggest that ALDH2 deficiency worsens AILI by promoting oxidative stress, enhancing the ALOX12-12-HETE signaling pathway.

Discussion: When ALDH2 activity is impaired, the overdose of APAP triggers lipid peroxidation. In *ALDH2*2* mice, reduced GSH may impair the detoxification of the toxic metabolite N-acetyl-4-benzoquinone imine (NAPQI), leading to sustained reactive oxygen species production and accumulation of toxic aldehydes like MDA. This oxidative stress and impaired aldehyde clearance state (via low ALDH2) further suppress reamining ALDH2 activity, aggravating mitochondrial structural damage. Proteomics and metabolomics revealed that ALDH2 deficiency leads to significant upregulation of lipid-peroxidation enzyme ALOX12 in the arachidonic acid metabolism pathway, as well as its product 12-HETE, which can trigger inflammation and cell death. From the results, it's proposed that the ALOX12-12-HETE axis represents pathological mechanisms linking ALDH2 deficiency, microcirculatory disturbance, and exacerbated AILI. Accordingly, these results provide novel insights into the pathogenesis of APAP-induced liver injury.

Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

Conflict of Interest

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P-360

Withdrawn

Withdrawn

P-361

Impact of Environmentally Induced Hypothermia on Fentanyl and Norfentanyl Pharmacokinetics Following Intravenous Administration to Wistar Rats

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Abstract

Introduction: Fentanyl, a synthetic opioid with potent analgesic and sedative properties, is widely administered in intensive care. Many critically ill patients present with, or develop, hypothermia driven by multifactorial disturbances such as sepsis, trauma, circulatory shock, or environmental exposure. Because reduced core temperature affects perfusion, metabolism, and clearance, hypothermia may meaningfully alter fentanyl disposition, although these effects remain insufficiently defined.

Objectives: This study investigates how moderate hypothermia (MH) and severe hypothermia (SH) in rodents alter the pharmacokinetics of fentanyl and its primary metabolite, norfentanyl, compared with normothermic controls.

Methods: 24 male Wistar rats, 373-534 g body mass and 18–20 weeks old, were divided into three groups and subjected to different environmental temperatures: normothermic controls (37 °C, *n* =7), MH (30 °C, *n*=9), and SH (27 °C, *n*=6). Fentanyl (10 µg/kg) was administered via a short intravenous infusion, and serial blood samples were collected at 5, 10, 15, 20, 30, 40, 50 and 60 min post-intervention. Serum fentanyl and norfentanyl concentrations were determined by liquid chromatography-electrospray ionization-tandem mass spectrometry (LC-ESI-MS/MS). Pharmacokinetic parameters were calculated using Phoenix WinNonlin software.

Results: Maximum fentanyl concentration increased in both MH and SH groups, reaching statistical significance only under severe hypothermia ($p < 0.001$). The area under the concentration-time curve (AUC) increased markedly ($p = 0.009$) in SH group, indicating increased overall drug exposure. Clearance decreased by 44% ($p = 0.010$), and volume of distribution in steady state (V_{ss}) was reduced in both groups (SH: $p < 0.001$, MH: $p = 0.029$), reflecting impaired drug elimination under severe hypothermic conditions. For norfentanyl, exposure increased significantly in the SH group across all parameters, whereas in the MH group increases were limited to C_{max} and $AUC_{0\text{-}t}$.

Discussion: The data demonstrate that even short-term moderate and severe hypothermia substantially modify fentanyl and norfentanyl pharmacokinetics in rats, primarily through reduced clearance and altered distribution. By examining hypothermia in the absence of major systemic pathology and by quantifying both parent drug and its principal metabolite, this study provides a mechanistic foundation for understanding temperature-related variability in fentanyl disposition and highlights the need to consider core body temperature as a significant covariate in opioid dosing

regimens in the perioperative and critical care setting. These findings should be interpreted with several important limitations in mind. The experiments were performed exclusively in male Wistar rats, restricting extrapolation across species and potentially overlooking sex- and age related variation in thermoregulation, hepatic perfusion, and CYP3A function. The 60-minute sampling window also failed to capture the terminal elimination phase - most notably for norfentanyl - thereby limiting full characterization of disposition kinetics. In addition, the absence of tissue concentration data precluded evaluation of pharmacokinetic–pharmacodynamic relationships in target organs such as the brain or lung.

Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

P-362

Methanol Poisoning Outbreak in Brazil: A Case Series and the Role of a Regional Poison Control Center in Clinical and Analytical Management

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Abstract

Introduction: Methanol poisoning is a major public health concern, particularly during outbreaks linked to adulterated beverages or contaminated fuel ethanol. In such scenarios, early recognition is challenging and requires integration of clinical suspicion, toxicological surveillance, and analytical confirmation. Poison control centers, when supported by clinical toxicology laboratories, are uniquely positioned as sentinel systems, bridging emergency medicine, public health response, and forensic investigation.

Objectives: To describe a series of suspected methanol poisoning cases managed by the Campinas Poison Control Center (CIATox-Campinas) during a large-scale Brazilian outbreak, characterizing the integrated role of clinical triage, analytical confirmation, and forensic interaction in outbreak detection and response.

Methods: A retrospective study was conducted using DATATOX, the institutional database of CIATox-Campinas, including cases recorded between September 2025 and January 2026. Cases were stratified into two groups: (I) externally referred samples submitted for analytical confirmation and (II) cases initially evaluated by the CIATox clinical team and subsequently referred for confirmatory testing. Group allocation reflected routine clinical workflow rather than a predefined design. Methanol and formic acid were quantified in whole blood by GC-FID using two analytical columns (BAC1 and BAC2). Demographic, clinical, and epidemiological variables were analyzed, and national surveillance data were used to contextualize the outbreak.

Results: Nationwide, 890 suspected and 73 confirmed methanol poisoning cases were reported, with 22 deaths; Sao Paulo was the main epicenter (578 suspected; 50 confirmed). Approximately 1,500 vials of fomepizole and 4,806 units of ethanol were distributed during the outbreak. At CIATox-Campinas, the index case presented high-anion-gap metabolic acidosis (pH 6.9–7.2), visual impairment, and CNS depression; methanol concentrations ranged from 10 to 150 mg/dL, with a mean formic acid concentration of 320 mg/L. Group I comprised 559 cases (724 analyses), with 10 positives (1.7%); Group II comprised 58 cases (75 analyses), with 12 positives (20.6%). Among Group II positives, outcomes were classified as fatal (n=7), severe (n=3), or moderate (n=4) according to the Poison Severity Score; 14 hospitalizations were recorded, including 10 ICU admissions. Epidemiological investigation identified a common exposure to adulterated alcoholic beverages, triggering a federal alert (SAR) and coordinated response. Forensic analysis traced the source to fuel-grade ethanol diverted for illicit beverage production, leading to the seizure of 17,700 adulterated items.

Discussion: The higher positivity rate in Group II compared with Group I (20.6% vs. 1.7%) likely reflects earlier sampling within the methanol detectability window, as externally referred cases were often collected after partial metabolism. These findings highlight the value of structured clinical-analytical triage in increasing pre-test probability and optimizing laboratory resource use. Integration of clinical assessment, GC-FID confirmation, and epidemiological investigation enabled early outbreak detection and supported both public health and forensic responses, contributing to the establishment of permanent funding and accreditation of poison control centers within the Brazilian Unified Health System (SUS). Overall, this experience reinforces the role of poison control centers, supported by clinical toxicology laboratories, as essential sentinel structures for national preparedness against chemical threats.

Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

P-363

PEth Recovery Pilot Study: The Effects of Time and Temperature on Cellular Localization of PEth

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Abstract

Introduction: Phosphatidylethanol (PEth) is a highly sensitive and specific biomarker of alcohol consumption, and important in clinical and forensic settings to investigate recent ethanol exposure and/or chronic use. PEth is frequently used to verify abstinence/use in transplant candidates. PEth positivity in individuals can have serious clinical and legal consequences. Consequences include clinically - transplant patient eligibility and delisting, false accusations for individuals with alcohol use disorder; and forensically - false accusations of vulnerable populations and child custody disputes.

PEth is formed in the red blood cell membrane from phosphatidylcholine and ethanol when present. The two predominant species, PEth 16:0/18:1 and PEth 16:0/18:2, account for more than 60% of the total PEth in blood. It is known that fortified incorporation of PEth that's fortified into whole blood is different than when compared to endogenous cell membrane content, hence recovery studies in extraction procedures have not been extensively studied.

The incubation time and temperature may affect the cellular location of PEth and subsequent adsorption by the RBC membrane. Inadequate membrane integration of exogenous PEth in calibrator preparation may increase percent recovery of PEth when compared to PEth incorporated into the plasma membrane, thus affecting the accuracy of PEth quantitation.

Objectives: To evaluate the effect of time and temperature on cellular localization of exogenous PEth.

Methods: PEth metabolites (16:0/18:1 and 16:0/18:2) were quantified in whole blood samples using a modified previously published method. Theoretical calibrators (5, 10, 20, 100, 200, 500ng/mL) were prepared by spiking PEth metabolites into isopropanol. Whole blood was prepared from PEth negative pooled blood collected within 24hours of venipuncture and stored at 4ºC overnight. The blood was fortified to 200ng/mL PEth and allowed to incubate for 1, 4, 6 and 24hours at 23ºC, 5ºC and 37ºC. At the end of the incubation period, each pool was aliquoted for analysis in triplicate. Separate aliquots were centrifuged; cell and plasma fractions were separated and analyzed. Storage containers were also evaluated (silanized vs non-silanized glass) and potential in vivo metabolism (NaF/NaO vs K_2EDTA).

Briefly, 100mcL of sample was aliquoted into cells of 96-well plate, 100mcL isopropanol containing 25mcL of PEth16:0/18:1-d5 and PEth16:0/18:2-d5 (internal standard, 25ng/mL) was added. Mix gently, then 600mcL isopropanol was added and thoroughly mixed, followed by 700mcL heptane. The plate was sealed, mixed and then centrifuged. The organic layer (1000mcL) was transferred to a clean plate and evaporated to dryness. The residue was reconstituted with 100mcL of methanol: isopropanol (1:1 v/v).

Results: PEth concentrations equilibrated for 24 hours at room temperature and refrigerated were consistent with PEth equilibrating with the red blood cell membrane. PEth concentrations equilibrated for 1, 4, or 6 hours at room temperature or refrigerated were comparable to the solvent-based matrix. PEth concentrations equilibrated at body temperature were inconclusive. No differences were observed between storage tube conditions (silanized vs non-silanized glass tubes and sodium fluoride and sodium oxalate tubes vs potassium EDTA).

Discussion: PEth calibrator and control blood control materials need to be equilibrated at room temperature or refrigerator temperature and for at least 24 hours before use to ensure that exogenously added PEth is equilibrated with the red blood cell membrane.

Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

P-364

Withdrawn

Withdrawn

P-365

Changing Patterns of Illicit Drug Seizures in South Korea in 2025: Expansion of Ketamine, Phencyclidine Analogues, and Anesthetic-Related Substances

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Abstract

Introduction: Illicit drug markets in South Korea have rapidly diversified, with persistent methamphetamine abuse and the increasing emergence of arylcyclohexylamines, synthetic cannabinoids, semi-synthetic cannabinoids, and anesthetic-related substances. Based on nationwide seized-drug casework examined by the National Forensic Service (NFS), this study describes major analytical findings and emerging drug trends observed in 2025.

Objectives: This study aimed to characterize illicit drug seizure trends in South Korea in 2025, focusing on changes in traditional drugs, ketamine and phencyclidine analogues, synthetic and semi-synthetic cannabinoids, and emerging prescription or anesthetic-related substances. The findings are intended to support forensic preparedness, early warning, and evidence-based regulatory responses.

Methods: Seized drug evidence submitted to the NFS in 2025 was reviewed using aggregated national casework statistics and detailed classifications by substance, physical form, and age group when available. Qualitative confirmation was performed using validated forensic analytical workflows, including gas chromatography–mass spectrometry, liquid chromatography–tandem mass spectrometry, and nuclear magnetic resonance spectroscopy when structural elucidation was required.

Results: In 2025, the NFS recorded 78,432 seized-item drug examinations, and 31,466 drug-positive seized-item classifications were reviewed by physical form. Powders were the most common form, followed by syringes and plant materials, reflecting the continued predominance of methamphetamine and the increasing contribution of ketamine and phencyclidine analogues. Methamphetamine remained the leading traditional illicit drug, while ketamine exceeded cannabis and opium poppy in major age-classified seizure categories. Synthetic cannabinoids remained prominent, with 25 compounds and 3,949 detections, although the total decreased from 2024. Their structures continued to be dominated by MDMB- and fluoro-substituted analogues, whereas ADB-type analogues declined. Semi-synthetic cannabinoids decreased compared with 2024, but continued structural modification was suggested by detections of tetrahydrocannabiphorol and hexahydrocannabiphorol. Phencyclidine analogues increased and diversified around 2-oxo arylcyclohexylamine scaffolds, including newly emerging PCP-related analogues. Other psychotropic substances rose sharply, mainly driven by etomidate and medetomidine, suggesting a shift in anesthetic-type substance misuse. Teenagers showed a particularly high proportion of synthetic cannabinoid detections, exceeding methamphetamine within this age group.

Discussion: The 2025 seizure profile demonstrates both continuity and transformation in South Korea's illicit drug landscape. Methamphetamine remains entrenched, but ketamine, phencyclidine analogues, synthetic cannabinoids, and anesthetic-related substances are reshaping the market. The rapid appearance of structurally modified analogues, changing physical forms such as powders and e-liquids, and youth-associated synthetic cannabinoid use indicate the need for proactive surveillance, continual spectral library updates, early acquisition of reference standards, and coordinated forensic–regulatory early warning systems.

Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

P-366

False Positives as Barriers to Justice: Evaluating the Reliability of Presumptive Drug Tests

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Abstract

Introduction: Presumptive drug tests used in law enforcement, originally designed for efficient field use, have recently been found to yield varying color changes that result in false positives. False positives from these presumptive drug tests raise serious concerns about their role as preliminary evidence in drug-related arrests and legal prosecutions. Common field kits utilize colorimetric tests that yield visual color changes when reagents react with specific functional groups but do not provide definitive compound identification, serving only as a preliminary indication of possible drug presence. Although presumptive field tests were originally intended as a preliminary indicator, these kits are often misinterpreted as proof of possession.

Objectives: The objective of this study was to directly compare and evaluate the cross-reactivity of commercial presumptive kits against common household and over-the-counter (OTC) compounds to quantify their forensic limitations in criminal investigations.

Methods: The analytical performance of multiple law enforcement drug detection kits was evaluated following the manufacturer's recommended protocol to assess their reliability. A false positive was determined using color charts and/or descriptions provided by the kit manufacturer and RAL color codes were assigned to each observed colorimetric response. All tests were observed, evaluated, and photographed by three independent evaluators in both sunlight and ambient lab light, and against neutral color backgrounds.

Results: The study identified numerous false positive results across multiple drug screening kits. Specifically, the NARK II Presumptive Narcotics Test Pouches produced 26 false positives, and 11 ambiguous results; NIK Test Pouches yielded 16 false positives, and 2 ambiguous results; NARK Field Test Tubes showed 10 false positives, and 5 ambiguous results; and F-1 fentanyl/opioid detection wipes resulted in 5 false positives results.

Discussion: Select commercially available presumptive drug testing systems were evaluated against a panel of pharmaceuticals/OTC compounds, such as ibuprofen and diphenhydramine, and common household materials, including flour and baking soda. Resulting outcomes were categorized as false positive, ambiguous, or negative, with corresponding RAL color codes recorded. False-positive responses were observed for multiple non-target compounds across several testing systems, with variability in both colorimetric response and compound specificity. The high frequency of false positives and ambiguous results across all tested platforms reveals a systemic forensic vulnerability that jeopardizes the accuracy of drug-related investigations.

Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

P-367

Extracellular Particle Dynamics and Lipidomic Alterations Following Nandrolone Administration: Toward Novel Biomarkers for Anti-Doping

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Abstract

Introduction: Conventional anti-doping analysis for anabolic steroids (AAS) such as nandrolone primarily relies on detecting drugs and their metabolites. However, these approaches are limited by the complexity of their metabolism and excretion, making detection challenging in some cases. Therefore, complementary biomarkers for detecting AAS misuse are needed. AAS alter cellular responses via androgen receptor signaling, which also affects lipid metabolism. Extracellular particles are formed through endosomal pathways, and their biogenesis is influenced by membrane lipid composition and properties. Thus, AAS-induced changes in lipid metabolism seem to remodel membrane environments and affect extracellular particle formation or secretion. These changes are expected to serve as complementary indicators for doping assessment.

Objectives: This study aimed to evaluate changes in extracellular particle dynamics following nandrolone administration and their association with lipid metabolic profiles, to assess their potential as novel biomarkers for anti-doping purposes.

Methods: Male Wistar rats (6 weeks old) were divided into three groups (n = 5 per group):control (vehicle), low-dose nandrolone (2 mg/kg/week), and high-dose nandrolone (10 mg/kg/week). Nandrolone was administered subcutaneously once weekly for two weeks. Plasma was collected on Day 14 and stored at −80°C. Extracellular vesicles were isolated by ultracentrifugation and analyzed using nanoparticle tracking analyser to assess particle concentration and size parameters. Lipidomic analysis was performed on plasma using liquid chromatography–quadrupole time-of-flight mass spectrometry. Statistical analysis included the Kruskal–Wallis test with Dunn's post hoc test and multivariate analysis.

Results: The mean extracellular particle concentrations were approximately 4.3×10^{10}, 4.1×10^{10}, and 1.6 × 1010 particles/mL in the control, low-dose, and high-dose groups, respectively. A significant decrease was observed in the high-dose group compared with both the control and low-dose groups ($p < 0.05$). No significant differences were observed in particle size-related parameters. Lipidomic analysis revealed group-dependent molecular changes with clear separation in multivariate analysis. MS/MS suggested that some discriminating features were associated with neutral lipids, including diacylglycerols.

Discussion: Diacylglycerols (DAGs) are cone-shaped lipids that influence membrane curvature and are involved in endosomal membrane budding and vesicle formation. They are also known to regulate signaling pathways, including protein kinase C, and contribute to the control of extracellular particle secretion. The formation and release of extracellular particles depend on the proper balance and localization of DAGs. However, alterations in lipid composition may disrupt this balance, leading to impaired membrane curvature formation and dysregulated signaling. Consequently, these changes may reduce the efficiency of vesicle biogenesis and secretion. The observed decrease in extracellular particle concentration in this study may reflect these combined effects. These findings suggest that combined changes in extracellular particle dynamics and lipid metabolic profiles following nandrolone administration may serve as potential novel biomarkers for doping evaluation. Future studies should investigate the temporal changes in extracellular particle dynamics and lipidomic profiles following AAS administration and determine their utility in complementing current anti-doping strategies.

Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

P-368

Role and Regulatory Mechanism of Th1/Th2 Imbalance-Mediated IFN-γ-GSK-3β-pTau Signaling Pathway in Ketamine-Induced Cognitive Dysfunction

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Abstract

Introduction: Chronic ketamine abuse can induce severe hippocampus-dependent cognitive dysfunction, yet the peripheral immune–central nervous system crosstalk mechanisms underlying this pathology remain incompletely elucidated.

Objectives: This study aims to systematically dissect the role of $CD4^+$T cell subset imbalance in ketamine-induced cognitive impairment, clarify the downstream regulatory mechanisms of the IFN-γ-GSK-3β-pTau signaling cascade, and provide new theoretical foundations and therapeutic targets for ketamine-related neurotoxicity.

Methods: Using a mouse model of repeated high-dose ketamine exposure in C57BL/6 mice, we integrated techniques including $CD4^+$T cell transcriptome sequencing, flow cytometry, qRT-PCR, ELISA, cell co-culture, siRNA knockdown, in vivo IFN-γ neutralizing antibody intervention, behavioral testing, Western blot, and histopathological staining to conduct both in vitro and in vivo mechanistic studies.

Results: Chronic ketamine exposure significantly skewed the peripheral $CD4^+$ T cell Th1/Th2 balance toward a Th1-dominant phenotype in mice, accompanied by a marked increase in the secretion of the Th1-type cytokine IFN-γ and a significant upregulation of its upstream driver, IL-12. Given that ketamine compromised blood–brain barrier integrity in mice, we confirmed that IFN-γ induced hippocampal glial cells secreted the chemokines CXCL9/CXCL10, which in turn recruited $CXCR3^+CD4^+$T cells into the hippocampus. In vivo neutralization of IFN-γ significantly reversed ketamine-induced impairments in spatial working memory and object recognition memory, and alleviated hippocampal neuronal pathological damage, reactive astrogliosis, and central neuroinflammation. Mechanistic studies further demonstrated that IFN-γ, via the neuronal IFNGR1 receptor, activated GSK-3β signaling and directly induced excessive phosphorylation of Tau protein at Ser396 in hippocampal neurons, whereas ketamine itself had no direct effect on inducing Tau phosphorylation.

Discussion: This study unveils for the first time that the Th1/Th2 imbalance-mediated IFN-γ-GSK-3β-pTau signaling axis represents a key immune–neural crosstalk mechanism underlying ketamine-induced cognitive dysfunction, providing a novel strategy for immune-targeted intervention against ketamine abuse-related neurocognitive impairment.

Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

Conflict of Interest

National Natural Science Foundation of China (32541068, 82030057, 82072111)

P-369

The Society of Forensic Toxicologists (SOFT) Professional Mentoring Program Year in Review: Assessment of 2025 Mentee/Mentor Outcomes and Trends

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Abstract

Introduction: As in many disciplines, mentoring is key in forensic toxicology, and it can happen at different stages of the professional career of a forensic toxicologist. To facilitate this opportunity, the Society of Forensic Toxicologists (SOFT) created and started the Professional Mentoring Program (PMP) in January 2020. This program allows SOFT members to seek guidance in specific areas of interest (including but not limited to development of leadership skills, technical development, research and publications). Additionally, the program also gives members the opportunity to contribute to the forensic toxicology community by mentoring other members of SOFT. The PMP program pairs two SOFT members at the beginning of the year, and the pair meets throughout the year to achieve the goals of interest defined by the mentee. Every year the PMP committee conducts a review of the feedback provided by mentors and mentees for continuous improvement purposes.

Objectives: The goal of this study was to review the feedback provided by participants in 2025 and assess general trends.

Methods: At the end of 2025, mentors and mentees were invited to complete a form and comment on their satisfaction regarding meeting the original goals established in conjunction with their mentor, resources provided, and the overall structure of the program.

Results: We received 32 individual responses to the survey out of 69 PMP participants (including mentors and mentees). The development of interpersonal/leadership skills and technical development were indicated as the most valued outcomes of the program. In 2025, 53% of the respondents indicated that the mentee's progress in developing interpersonal and leadership skills met or exceeded expectations (compared to the beginning of the program). Regarding technical development, the mentee's progress met or exceeded expectations according to 43% of the respondents. Transfer of knowledge and expanding professional networks were considered major or significant benefits from the program for 87% of participants. The most valuable resource was ToxTalk as indicated by 81% of participants, followed by the kickoff webinar with 66% of participants. However, most participants did not utilize on-demand activities. In terms of program expectations, the program met or exceeded

the expectations of 91% of participants. For future activities, most of the responses indicated high interest in leadership activities/resources followed by laboratory workflow development and improvement, activities at the SOFT annual meeting, and facilitated activities for the mentor-mentee pair.

Discussion: Most of the results are consistent with the feedback received in previous years. The development of interpersonal and leadership skills and technical development remained the most significant outcomes for the participants. Transfer of knowledge and expanding professional networks remained the main benefits of the program. Interestingly, in 2025, ToxTalk was indicated as the most valuable resource, in contrast to 2024. The results indicated a high level of satisfaction with the program in 2025. Regarding resources, current changes are being considered to better meet participants' needs and expectations based on recent feedback. Overall, the PMP continues to meet or exceed the expectations of participants and to be a key program for the forensic toxicology community.

Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

P-371

Scenario-Based Learning for Forensic Case Reconstruction Using Toxicological Findings and Case Data: An Educational Study Among Policing College Students

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Abstract

Introduction: Forensic toxicology findings are most useful when interpreted in relation to the wider investigative context. In policing education, students must understand how toxicological results connect with crime scene observations, intelligence information, and other forensic reports. This study presents a scenario-based learning model designed to help policing college students reconstruct basic toxicological cases using integrated forensic and case-based data.

Objectives: This study aimed to evaluate whether scenario-based toxicological cases can improve students' ability to review case information, interpret toxicological findings, integrate supporting forensic evidence, and develop logical case reconstructions.

Methods: Basic forensic toxicology scenarios were developed as structured case packages. Each package included a crime scene description, intelligence information, forensic toxicology report, DNA report, fingerprint report, and relevant case background. The scenarios represented basic toxicological patterns suitable for policing college students and focused on the relationship between suspected exposure, scene observations, toxicological findings, and supporting forensic evidence.

Students were divided into 18 groups, and each group was assigned a case package. They were required to examine the materials, identify key findings, compare toxicological results with crime scene and intelligence information, consider the contribution of DNA and fingerprint evidence, and prepare a basic reconstruction of the case. Group performance was evaluated using a structured assessment form covering three areas: integration of case data into a coherent report, formulation of interpretive opinions based on a crime scene reconstruction model with reference to the provided reports, and overall understanding of the case as a connected investigative and scientific narrative.

Results: The model encouraged students to move beyond describing isolated laboratory findings toward interpreting toxicology results within a broader investigative framework. Group reports showed improved ability to combine crime scene observations, intelligence information, toxicological findings, DNA results, and fingerprint evidence into structured case reports. Stronger submissions explained the possible sequence of events, identified the likely role of the toxic substance, and supported conclusions by referring directly to the supplied forensic reports.

The assessment also identified areas requiring further development. These included distinguishing confirmed analytical findings from interpretive opinions, recognizing the limitations of toxicological evidence, and avoiding unsupported conclusions about intent, timing, or causation. These findings indicate that structured scenario-based exercises can reveal both strengths and gaps in students' forensic reasoning.

Discussion: This study demonstrates the value of scenario-based learning in teaching policing college students how toxicological findings may contribute to forensic case reconstruction. By combining investigative information with toxicology, DNA, and fingerprint reports, the model reflects the multidisciplinary nature of real forensic casework. The structured assessment form supports competency-based learning by providing a measurable approach for evaluating evidence integration, reconstruction logic, report writing, and case interpretation. This approach may strengthen students' forensic awareness and improve their readiness to understand expert reports, communicate with forensic laboratories, and use toxicological evidence appropriately during investigations.

Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

P-372

Detection of N-Ethylamphetamine in Methamphetamine-Positive Casework

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Abstract

Introduction: N-ethylamphetamine (NEA), also known as Etilamfetamine, and formerly sold under the brand names Apetinil and Adiparthrol, is a Schedule I synthetic phenethylamine structurally related to methamphetamine (MET) and amphetamine (AMP), historically used clinically as an appetite suppressant. It is typically not targeted in routine forensic toxicology analyses. In 2025, the San Francisco Office of the Chief Medical Examiner (OCME) implemented LC-QTOF-MS for routine casework, expanding the scope of testing to nearly 1000 analytes, including NEA and MET-related compounds and metabolites. Following NEA inclusion into routine testing, a notable increase in NEA was observed among MET-positive cases. As NEA was not captured with prior targeted methods, its prevalence and relationship to co-detected analytes in these populations are unknown, prompting a systematic review of MET and NEA-positive casework.

Objectives: Determine the prevalence of NEA in routine casework and characterize its relationships, including co-detections and concentration ratios between NEA, MET, and MET-related compounds across specimen types, case types, and demographic groups.

Methods: All driving-under-the-influence, sexual assault, 11550 (public intoxication), and postmortem casework from January 1, 2025 through March 31, 2026, were retrospectively reviewed for NEA, MET, AMP, 4-hydroxymethamphetamine (4-OH), ephedrine/pseudoephedrine (EPP), and norephedrine/norpseudoephedrine (NOR) in blood and urine (n > 1500 cases). Blood and urine results for the six target analytes were consolidated by case, and analyte-to-NEA concentration ratios were computed for each specimen type. Descriptive statistics (N, mean, median, min, max, Q1, Q3) were generated overall and stratified by specimen type, case type, and demographic variables (gender, race, age group).

Results: NEA was detected in 731 total cases (275 cases in blood only, 604 cases in urine only; 148 cases in both blood and urine). The median NEA concentration was 32 ng/mL in blood and 170 ng/mL in urine. NEA was detected in 275 of 895 MET-positive blood cases (30.7%). NEA was detected in combination with MET in blood 100% of cases and never detected in combination with AMP alone.

The median blood (and urine) concentration ratios for NEA were as follows: MET/NEA 21.9 (117.5); AMP/NEA 2.89 (13.8); EPP/NEA 0.741 (0.066); NOR/NEA 0.229 (0.784); and 4-OH Meth/NEA 0.159 (2.71).

NEA was observed across all demographic groups examined, with detections seen in both sexes, across adult age ranges, and across reported race categories. Notably, as age increased cases tended to have higher median blood concentrations but lower median urine concentrations.

Discussion: The adoption of LC-QTOF-MS expanded the analytical scope sufficiently to surface NEA as a recurring co-detection in MET/AMP-positive casework, a finding that would not have been captured with prior targeted methods. The consistent pattern of MET/NEA prevalence suggests NEA may be present as an impurity or by-product of illicit MET, though this interpretation relies on limited evidence; and its origins remain uncertain; alternative sources, including adulterant origins cannot be excluded. Additionally, NEA can be N-dealkylated to form AMP, which may further impact interpretative assessments of MET to AMP expected ratios. Continued surveillance may be beneficial to track prevalence and to clarify the toxicological significance of NEA in MET-positive casework.

Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.

P-373

Automated LC-MS/MS Data Reporting Workflow using mzML and R for Urine Drug Screening and Quantitative Confirmation

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Abstract

Introduction: Monitoring medication compliance in mentally disordered offenders is important for effective rehabilitation and the reduction of recidivism. Although liquid chromatography-tandem mass spectrometry (LC-MS/MS) is widely used as a primary analytical technique for forensic urine drug testing, manual data evaluation using vendor-specific software can limit laboratory efficiency and introduce subjective variability and human error among forensic toxicologists.

Objectives: This study aimed to develop and validate a fully automated, vendor-neutral data reporting workflow that requires no specialized hardware. It focused on the standardization of data file formats using open-source mzML and the automation of reporting functions through R programming—specifically utilizing the highly efficient mzR package—to seamlessly improve objectivity and analytical throughput in urine drug screening and quantitative confirmation on standard laboratory computers.

Methods: Primary instrumental analysis and data acquisition were conducted using a Waters Xevo TQ-XS triple quadrupole mass spectrometer. An integrated analytical approach was implemented in two stages. In the initial screening stage, an automated detection system for 59 psychotropic drugs and metabolites was developed by applying logic-based criteria. In the confirmation stage, rigorous quantitative analysis for quetiapine and norquetiapine was established based on ANSI/ASB Standard 036, enforcing strict mathematical tolerances such as a ±25% ion ratio variance limit. The entire workflow was comprehensively validated using forensic urine samples from probationers in accordance with the U.S. FDA "Bioanalytical Method Validation Guidance for Industry." To evaluate true cross-platform versatility, raw binary data from Agilent, Shimadzu, and Thermo Scientific analyzers were also converted and processed.

Results: The automated system demonstrated analytical reliability by directly integrating pre-validated parameters (e.g., limit of detection, lower limit of quantification, linearity, precision, and accuracy) as objective algorithmic thresholds for data processing. By automatically flagging hidden matrix interferences, the workflow effectively removed the subjectivity and human error associated with manual reviews, thereby preventing falsely inflated concentrations and fortifying evidentiary integrity.

Discussion: This open-source workflow provides a standardized, vendor-neutral solution that may enhance the reliability and objectivity of analytical results without requiring advanced computing power. Its versatility successfully dissolves technical barriers imposed by proprietary formats, enabling streamlined data evaluation regardless of the instrument manufacturer and supporting its broad application for forensic toxicologists in medication compliance monitoring.

Disclosure

No, I, nor any member of my immediate family, has a financial interest to disclose.