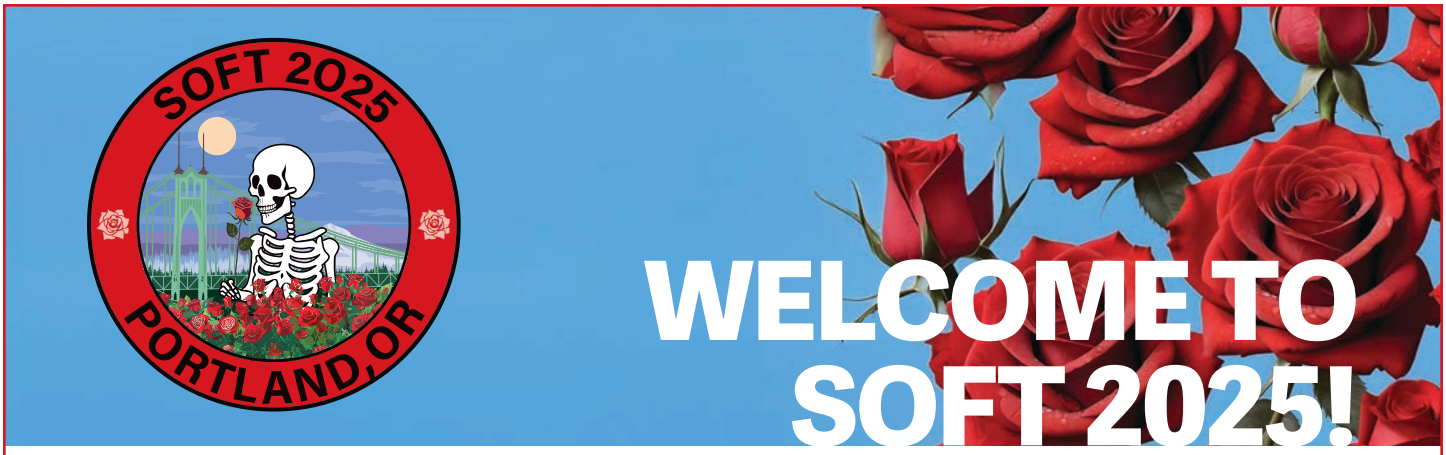
PROGRAM & ABSTRACTS



Dear SOFT 2025 Attendees,

Welcome to the 2025 Society of Forensic Toxicologists Annual Meeting! We are delighted to welcome you to beautiful Portland, Oregon, a city known for its creativity, natural beauty, and vibrant community. Whether you're joining us for the first time or returning for another year of connection and collaboration, we're so glad you're here.

As your hosts, we want to express our deepest appreciation to the outstanding Planning Committee. Their dedication, professionalism, and teamwork have made this meeting possible. It has been a true pleasure working alongside such a talented and passionate group. Their efforts have shaped an exceptional program filled with cutting-edge science, engaging workshops, and memorable social events.

We're especially excited about the robust workshop program and dynamic scientific sessions lined up this year. From hands-on training opportunities to presentations showcasing the latest advancements in forensic toxicology, the content reflects the innovation and expertise that define our field. We're confident you'll find inspiration, practical insights, and plenty of opportunities to engage with colleagues and thought leaders.

We are grateful for the many exhibitors that continue to support SOFT and this year is no different. They are here to address our analytical needs and demonstrate the latest technology to help us achieve our goals.

As in last year's meeting, we will be offering free yoga to begin your day in a centered way. For those who enjoy getting outside early in the morning, the Fun Run route is a great way to run (or walk) along and across the Willamette River, taking in different views of the city and its historic bridges.

The offsite event at The Redd on Salmon Street will be a unique venue to unwind with fellow attendees, savor great food, and enjoy entertainment by AWOL Dance Collective aerialists. How many of you can say you've attended a dinner while performers dazzle us from the ceiling?!

We hope you take full advantage of the opportunities to learn, share, and grow throughout the week. And while you're here, we encourage you to explore Portland's unique charm, from its lush parks and eclectic neighborhoods to its renowned food scene and welcoming spirit.

Thank you for being part of SOFT 2025. We look forward to an inspiring and enjoyable week with all of you!

Warm regards,
Sara Short & Amy Miles
SOFT 2025 Hosts

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Meeting Host

SARA SHORT
Meeting Host

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Chris Heartsill

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Amy Miles

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Delisa Downey

YOUNG FORENSIC TOXICOLOGISTS
Elisa Shoff

Sunday, October 26, 2025	Time	Place
Registration	10am-5pm	Ginkoberry Concourse
NSC-ADID Meeting	11am-2pm	E147-148
NLCP Inspector Training	2-6pm	E141-144
YFT Symposium	5-8pm	F149-152

Monday, October 27, 2025	Time	Place
Yoga	6-7am	Hyatt-Columbia 1, 3rd Fl.
Breakfast WS Participants Only	7-8:30am	Portland Ballroom 255-256
Registration	7am-6pm	Ginkoberry Concourse
Student Enrichment Program	9am-2pm	E147-148
WS 1: Mental Health	8am-12pm	F151
WS 2: Smoke Shops	8am-12pm	F150
WS 3: Pediatric Testing	8am-12pm	E141-144
WS 4: Methods I	8am-12pm	E145-146
ABFT Exam Committee	8am-5pm	B116
Break WS Participants Only	10-10:30am	Pre-Function E
Agilent Lunch and Learn	12-1:30pm	B110-112
Waters Lunch and Learn	12-1:30pm	B117-119
Thermo Fisher Lunch and Learn	12-1:30pm	B113-114
WS 5: Methods II	1:30-5:30pm	E145-146
WS 6: Postmortem Drug Screen	1:30-5:30pm	F151
WS 7: Alternative Matrices	1:30-5:30pm	F150
WS 8: Prescription Drugs	1:30-5:30pm	E141-144
Break WS Participants Only	3:30-4pm	Pre-Function E
NPS Committee Meeting	5:30-6:30pm	D133-134
Drugs & Driving Committee Mtg.	5:30-6:30pm	D139-140

Tuesday, October 28, 2025	Time	Place
Yoga	6-7am	Hyatt-Columbia 1, 3rd Fl.
Breakfast WS Participants Only	7-8:30am	Portland Ballroom 255-256
AAFS Steering Committee	7-8:30am	D135
Registration	7am-6pm	Ginkoberry Concourse
SOFT Board Meeting	8am-12pm	D133-134
WS 9: Stimulants	8am-12pm	E141-144
WS 10: MS-Based Forensic Tox	8am-12pm	E145-146
WS 11: Data Analysis	8am-12pm	F150
WS 12: Roadside Oral Fluid	8am-12pm	F151
ABFT Exam	8am-12pm	B116
Break WS Participants Only	10-10:30am	Pre-Function E
Agilent Lunch and Learn	12-1:30pm	B110-112
SCIEX Lunch and Learn	12-1:30pm	B117-119
Restek Lunch and Learn	12-1:30pm	B113-114
ABFT Board Meeting	12-5 pm	D137
WS 13: Expert Testimony	1:30-5:30pm	E141-144
WS 14: Portland Postmortem	1:30-5:30pm	E145-146
WS 15: DOTS	1:30-5:30pm	F150
WS 16: NPS Drug Ecosystem	1:30-5:30pm	F151
Break WS Participants Only	3:30-4pm	Pre-Function E
Oral Fluid Committee	5:30-6:30pm	D139-140
Publications Committee	5:30-6:30pm	D135

Tuesday, October 28, 2025	Time	Place
Postmortem Committee	5:30-6:30pm	D133-134
Welcome Reception	6:30-9:30pm	Exhibit Hall D
Elmer Gordon Forum	8:30-10pm	Oregon Ballroom
MilliporeSigma Nite Owl	10pm-12am	Oregon Ballroom

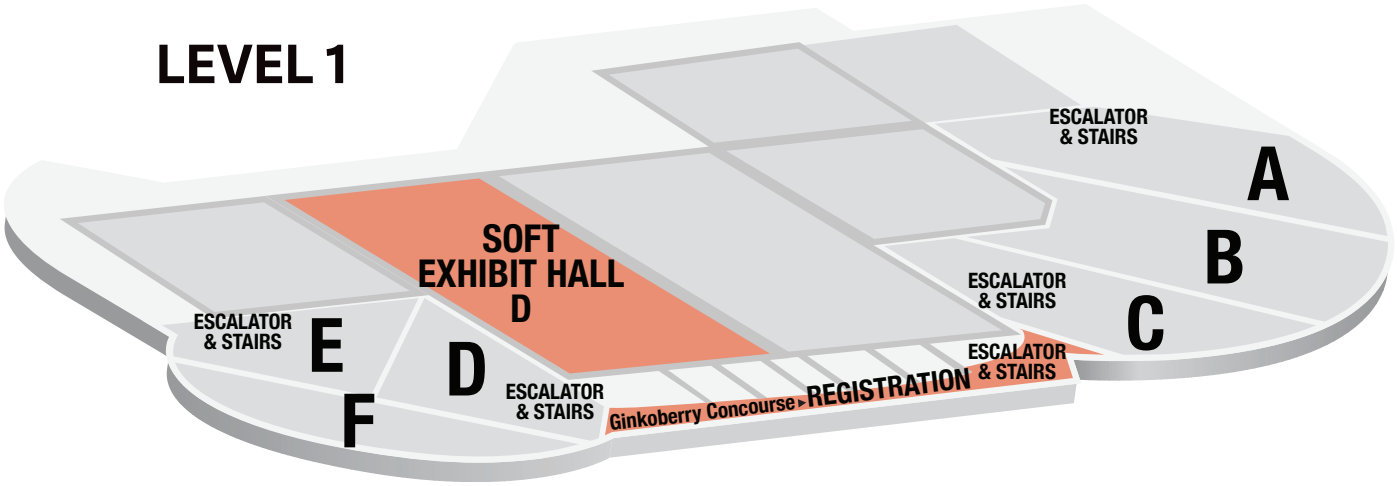
Wednesday, October 29, 2025	Time	Place
Yoga	6-7am	Hyatt-Columbia 1, 3rd Fl.
First-Time Attendee Breakfast	7-8am	Portland Ballroom 251
Breakfast	7-8:30am	Portland Ballroom 255-256
Poster Presentation Prep	7-11am	Exhibit Hall D
Registration	7am-5pm	Ginkoberry Concourse
Plenary Speaker	8-9am	Portland Ballroom
Session 1: Cannabinoids & Clinical	9-10am	Portland Ballroom
Exhibit Hall	9:30am-4pm	Exhibit Hall D
Break	10-10:30am	Exhibit Hall D
Session 2: Analytical & Human Perf.	10:30am-12pm	Portland Ballroom
Lunch with Exhibitors	12-2pm	Exhibit Hall D
Poster Session	12-2pm	Exhibit Hall D
SOFT 2026 Planning Committee	12:30-1:30pm	D139-140
Session 3: NPS and Postmortem	2-3:30pm	Portland Ballroom
Break	3:30-4pm	Exhibit Hall D
Session 4: Best Practice	4-5pm	Portland Ballroom
Off Site Event	6-10pm	The Redd

Thursday, October 30, 2025	Time	Place
Karla Moore Fun Run	6:30-8am	Convention Center Entrance
Breakfast	7-8:30am	Portland Ballroom 255-256
Poster Presentation Prep	7-11am	Exhibit Hall D
Registration	7am-1pm	Ginkoberry Concourse
Session 5: Kratom	8-10am	Portland Ballroom
Exhibitor Feedback Meeting	8:30-9:30am	Portland 256
Exhibit Hall	9:30am-3:30pm	Exhibit Hall D
Break	10-10:30am	Exhibit Hall D
Session 6: Drugs & Driving	10:30am-12pm	Portland Ballroom
Lunch with Exhibitors	12-2pm	Exhibit Hall D
Poster Session	12-2pm	Exhibit Hall D
DFC Committee	12:30-1:30pm	Portland Ballroom 251
Session 7: Awards	2-3pm	Portland Ballroom
Break	3-3:30pm	Exhibit Hall D
SOFT Business Meeting	3:30-5pm	Portland Ballroom
Happy Hour	6-7pm	Portland Ballroom Lobby
President's Banquet	7-8:30pm	Portland Ballroom
Live Band and Dancing	8:30pm-12am	Portland Ballroom

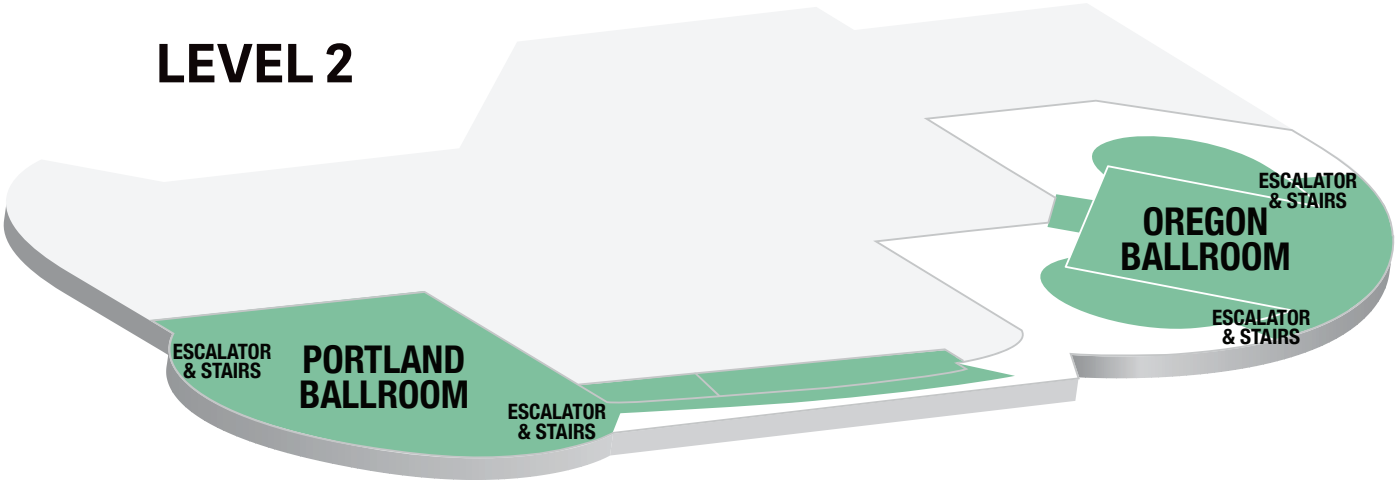
Friday, October 31, 2025	Time	Place
Breakfast	7-8:30am	Portland Ballroom 255-256
Session 8: Various Topics	8-10am	Portland Ballroom
Break	10-10:30am	Portland Ballroom 255-256
Session 9: NPS and Postmortem	10:30am-12pm	Portland Ballroom

OREGON CONVENTION CENTER MAP

LEVEL 1



LEVEL 2



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HEAR FROM THE BOARD OF DIRECTORS
AT THE SOFT BUSINESS MEETING
THURSDAY, OCTOBER 30, FROM 3:30-5:00 PM.

- OFFICER REPORTS
- AWARDS
- REGISTRATION RAFFLE

To everyone who reviewed Annual Meeting abstracts and JAT (Journal of Analytical Toxicology) Special Issue manuscripts: your expertise, thoughtful feedback, and on-time reviews shaped a rigorous scientific program and strengthened our journal. SOFT is better because of you. Thank you!

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SOFT Events Sponsored by Tier I Sponsors Include:

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KARLA MOORE FUN RUN/WALK SPONSOR – $250

Karla Moore Fun Run/Walk
Thursday, October 30 • 6:30–8:00 AM

ABOUT THE FUN RUN/WALK

Join us bright and early on Thursday, October 30 at 6:30 am for the Karla Moore Fun Run/Walk. Participants may run or walk the route; volunteers with signage will be along the course to help direct you. There are no awards—just bragging rights! Your $30 donation includes a Fun Run shirt and lapel pin. Proceeds are donated to the American Cancer Society in Dr. Moore's memory.

FUN RUN/WALK HISTORY

The original Tox 'N Purge run was created by Dr. Karla Moore in 1997 for the Salt Lake City meeting. In addition to her contributions to forensic toxicology and SOFT, Dr. Moore served as an officer in the United States Air Force. After her passing in 2008, the run was memorialized in her honor. Proceeds from the event are donated to the American Cancer Society in Dr. Moore's memory, and event expenses are supported by our SOFT exhibitors.

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NETWORKING OPPORTUNITIES

MORNING YOGA

Mon–Wed, October 27–29
6:00–7:00 AM

We're excited to host morning yoga sessions at this year's SOFT Meeting! Join us Monday, Tuesday, and Wednesday from 6–7 AM to start your day with relaxation and rejuvenation. Whether you're a seasoned yogi or new to the practice, all are welcome to participate. The instructor will have yoga mats on a first come first serve basis, or feel free to bring a travel mat or towel.

WELCOME RECEPTION

Tuesday, October 28
6:30–9:30 PM

Join us for the grand opening of SOFT's Exhibit Hall! Explore the exhibits, reconnect with colleagues, and enjoy the provided food and beverages. Don't forget to check out the Exhibit Hall Map in SOFT's Mobile App. This event is open to all SOFT attendees.

ELMER GORDON FORUM DESSERT RECEPTION

Tuesday, October 28
8:30–10:00 PM

An opening-night tradition: over dessert, join a lively, practical forum. Bring a topic, challenge, or question from your own lab—and help answer others. Come to ask, answer, or simply listen and leave with real-world takeaways. Open to all SOFT attendees.

MILLIPORESIGMA NITE OWL RECEPTION

Tuesday, October 28
10:00 PM–12:00 AM

End your evening at the Nite Owl Reception, hosted by our Tier II sponsor, MilliporeSigma. Open to all SOFT attendees.

NETWORKING OPPORTUNITIES

FIRST-TIME ATTENDEE BREAKFAST

Wednesday, October 29
7:00–8:00 AM

Is this your first time attending a SOFT meeting? Fantastic! Don't miss the chance to connect with the SOFT Board of Directors and the SOFT 2025 Planning Committee, as well as fellow newcomers at the First-Time Attendee Breakfast. Open to all First-Time Attendees.

AN EVENING OUT AT THE REDD

Wednesday, October 29
6:00–10:00 PM

Join us for an off-site evening with provided transportation, plus food and beverages on-site. Look up for graceful aerial performances overhead at intervals during the night. Don't miss the special highlight act at 8:30 pm! Open to all SOFT attendees.

KARLA MOORE FUN RUN/WALK

Thursday, October 30
6:30–8:00 AM

Join us for the Karla Moore Fun Run/Walk, a tradition that began in 1997 with the Tox 'N Purge run, created by Dr. Karla Moore. Proceeds benefit the American Cancer Society in her memory. Donation: $30 (includes a lapel pin & shirt).

PRESIDENT HEARTSILL'S RED ROSE BALL

Thursday, October 30
Cocktail Hour 6:00–7:00 PM
Dinner & Dancing 7:00 PM–12:00 AM

Celebrate our closing evening in the City of Roses with dinner, drinks, and dancing. It's a Red Rose–themed night from start to finish -- dress up and join in! Open to all SOFT attendees.

YFT SYMPOSIUM & PROFESSIONAL DEVELOPMENT FAIR

SUNDAY, OCTOBER 26
5:00-8:30 PM

Symposium: Join us for a night of professional networking designed for early-career toxicologists. Enjoy drinks and hors d'oeuvres while you connect with peers in your field. This event is exclusively for attendees aged 41 and under.

5:00–6:00 PM
Professional Development Fair

6:05–6:20 PM
Leo dal Cortivo Platform Winner 2024: Amanda Pacana

6:20–6:35 PM
Leo dal Cortivo Poster Winner 2024: Brianna Stang

6:40–7:00 PM
Icebreaker with the Board

7:00–7:30 PM
Future of Toxicology Panel

- **AI (LIMS): Tyler Devincenzi**
- **AI (QToF): Maria Sarkisian**
- **Automation: Luke Rodda**
- **Green Toxicology: Sabra Jones**
- **Staying Current with New Drugs: Kari Midthun**

7:30–8:30 PM
Open Discussion: Audience & Panel

Professional Development Fair: Representatives from various agencies, graduate programs and laboratories will be available to discuss opportunities for continuing education, professional training, certification, academic advancement and career opportunities.

HOSTED BY THE YOUNG FORENSIC TOXICOLOGISTS COMMITTEE

SOCIETY OF FORENSIC TOXICOLOGISTS
Young Forensic Toxicologists

PLENARY SPEAKER
JUDGE NAN G. WALLER

JUDGE NAN WALLER: ADVANCING JUSTICE THROUGH COMPASSION AND SCIENCE

8:00-9:00 AM
WEDNESDAY, OCTOBER 29

Judge Nan Waller has long been a transformative force in the judicial system, known for her tireless advocacy for individuals with mental health needs and her innovative approach to court reform. As the presiding judge of Multnomah County's mental health court and competency docket, she has redefined how courts can serve as gateways to treatment, dignity, and hope.

Recognizing that substance use often intersects with mental health challenges, she is championing the use of toxicology data to inform more accurate, timely, and compassionate responses within the courtroom. By incorporating scientific evidence into legal proceedings, Judge Waller aims to improve outcomes for individuals whose behavior may be influenced by undiagnosed or untreated substance use disorders.

Her leadership reflects a broader commitment to data-driven justice. Whether advocating for improved access to behavioral health services, co-chairing the Oregon Chief Justice's Behavioral Health Advisory Council, or supporting national initiatives to reform court practices, Judge Waller continues to push boundaries—ensuring that the justice system evolves to meet the complex realities of those it serves.

Judge Waller's work reminds us that justice is not static—it is a living system that must adapt, learn, and respond. Through her efforts to integrate forensic toxicology and mental health awareness, she is paving the way for a more informed, humane, and effective judicial process.

Journal of

Analytical Toxicology

The Journal of Analytical Toxicology (*JAT*) is the official journal for **The International Association of Forensic Toxicologists** (TIAFT), as well as the **Society of Forensic Toxicologists** (SOFT).

Publishing timely dissemination of scientific communications concerning analyses and interpretation of impairing and toxic substances in biological matrices with an emphasis on their applications to clinical and forensic toxicology.

JAT Metrics

Impact Factor: 2.6
JCR Rank: 60/111
CiteScore: 5.8
Cited Half-Life: 8.7

949,000+ ARTICLE READS IN 2024

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Showcase your research in front of a global audience

30

days average from submission to first decision

6

days average from final decision to online publication

WORKSHOP PROGRAM

PROGRAM COORDINATORS:
Dani Mata & Mary Lynn Heffington

MONDAY, OCTOBER 27
8:00 AM - 12:00 PM

WS01: From Crisis to Calm: Mental Health in Forensic Science Workplaces

Chairs: Kristen Burke, M.S., MBA & Sabra Jones, Ph.D.

WS02: The Rise of Smoke Shops & Commercial Sales of NPS

Chairs: Kayla N. Ellefsen, Ph.D. & Helen Chang, M.S.

WS03: A Closer Look at Pediatric Toxicology: Unraveling the Mysteries of Pediatric Testing

Chairs: Theresa Hippolyte, M.S., D-ABFT-FT & Lindsay Glicksberg, Ph.D.

WS04: Method Development: Foundations for the New Toxicologist, Part I

Chairs: Elisa N. Shoff, M.S., D-ABFT-FT & Wayne Lewallen, B.S.

MONDAY, OCTOBER 27
1:30 - 5:30 PM

WS05: Forensic Toxicology Validation: Navigating the Latest ANSI/ASB 036 Revisions: Part II

Chairs: Rebecca Wagner, Ph.D. & Robert Lockwood, Ph.D.

WS06: The "Postmortem Blood Drug Screen": Analytical & Managerial Approaches

Chairs: Carl E. Wolf, Ph.D. & Justin L. Poklis, B.S.

WS07: Choosing Wisely: How Alternative Biological Matrices Shape & Support Forensic Interpretation

Chairs: Eduardo de Campos, Ph.D. & Ana Pego, Ph.D.

WS08: Beyond the Pill Bottle: Prescription Drugs' Hidden Impacts on Driving, Work, & Life

Chairs: Kristen Burke, M.S., MBA & Sabra Jones, Ph.D.

WORKSHOP PROGRAM

TUESDAY, OCTOBER 28
8:00 AM - 12:00 PM

WS09: Stimulants & the Road Ahead

Chairs: Anisha Paul, M.S. & Vanessa Meneses, M.S.

WS10: Recent Advances in MS-based Forensic Toxicology: Achieving Efficiency, Selectivity, & Sensitivity

Chairs: Brittany Friedman & Laura Friederich

WS11: Data Analysis: Collection, Preparation & Visualization

Chairs: Brigitte Desharnais, Ph.D. & Sue Pearring, M.S.

WS12: Roadside Oral Fluid Testing Device Evaluation & Approval

Chairs: Curt E. Harper, Ph.D. & Mandi Mohr, M.S.

TUESDAY, OCTOBER 28
1:30 - 5:30 PM

WS13: "Can I Say That?" Mastering Expert Testimony in Forensic Toxicology

Chairs: Robert Johnson, Ph.D. & Kei Osawa, MFS

WS14: Death in Portland, Let Knowledge Service the City. Postmortem Toxicology—How to Complete a Thorough Investigation

Chairs: Craig N. Chatterton, Ph.D. & Luke N. Rodda, Ph.D.

WS15: Uniting Forensic Toxicology & Medical Toxicology to Implement the Drug Overdose Toxico-Surveillance (DOTS) Reporting Program

Chairs: Alex J. Krotulski, Ph.D. & Paul Wax, MD

WS16: Leveraging Large Language Models to Investigate the Novel Psychoactive Drug Ecosystem: Applications in Structural Prediction, Behavioral Analysis, & Cyber Surveillance

Chairs: Lynn M. Wagner, Ph.D. & Erin R. Wilfong, Ph.D.

WEDNESDAY, OCTOBER 29, 2025 MODERATORS: Aria McCall & Vanessa Meneses TOPIC: Cannabinoids & Clinical			
TIME	**#**	**TITLE**	**PRESENTER**
9:00-9:12 am	S-01	The Effect of Vaporized and Oral Δ8-Tetrahydrocannabinol vs Δ9-Tetrahydrocannabinol on Pharmacokinetics and Pharmacodynamics in Healthy Adults	Austin Zamarripa
9:12-9:24 am	S-02	Blood Cannabinoid Findings in Human Performance and Postmortem Casework from 2024-2025: Implications for Forensic Toxicology Laboratories	Glenna Brown
9:24-9:36 am	S-03	From Exposure to Evidence: Recent Casework Involving Cannabinoid Use and Adverse Events	Michelle Peace
9:36-9:48 am	S-04	Lipid Lifeboats: Capturing Fentanyl with Bioengineered Liposomes	Alaina Holt
9:48-10:00 am	S-05	Determination of neurotransmitter levels in rat brain following opioid-sedative exposure using liquid-chromatography quadrupole-time-of-flight mass spectrometry	Laerissa Reveil
10:00-10:30 AM: MORNING BREAK			

WEDNESDAY, OCTOBER 29, 2025 MODERATORS: Rusty Lewis & Kei Osawa TOPIC: Analytical Methods & Human Performance			
TIME	**#**	**TITLE**	**PRESENTER**
10:30-10:42 am	S-06	Translating UPLC-QTOF Screening from Research to Routine: Validation, Accreditation, and Implementation for Systematic Toxicological Analysis	Jari Rubbens
10:42-10:54 am	S-07	Impact of Stop-Limit Testing Practices at the Wisconsin State Laboratory of Hygiene… and Beyond?	Kayla Neuman
10:54-11:06 am	S-08	Impaired Driving Surveillance in Arkansas: Year One Review.	Mary Lynn Heffington
11:06-11:18 am	S-09	Alive and Somehow Behind the Wheel	Leanne Hazard
11:18-11:30 am	S-10	Impaired Driving Cases with Diphenhydramine	Nicholas Tiscione
11:30-11:42 am	S-11	Puff, Puff, Pass [the alcohol drug test?]: A Clinical Study Assessing Impacts of Vaped Ethanol on Ethanol Testing	Alaina Holt
11:42-11:54 am	S-12	Analysis of Confiscated Vaping Products from Public Schools across the Commonwealth of Virginia during the 2024-25 Academic Year	Meredith Buckmire
12:00-2:00 PM: LUNCH			

WEDNESDAY, OCTOBER 29, 2025 MODERATORS: Samantha Tolliver & Kayla Ellefsen TOPIC: NPS & Postmortem			
TIME	**#**	**TITLE**	**PRESENTER**
2:00-2:12 pm	S-13	The Emergence of the Designer Stimulants Fluorexetamine and 2-Fluoro-2oxo-PCE in Clinical Urine Specimens	Jillian Neifeld
2:12-2:24 pm	S-14	The Identification of N-Isopropyl Butylone in Postmortem Cases in Northern Virginia	Ashley Pluer
2:24-2:36 pm	S-15	Comprehensive Profiling and Characterization of NPS Drugs Using Trapped Ion Mobility-High Resolution Mass Spectrometry	Akshita Verma
2:36-2:48 pm	S-16	In vitro metabolism of N-piperidinyl etonitazene for forensic toxicology investigations	Sara Kuberski
2:48-3:00 pm	S-17	Postmortem Benzodiazepines 6-Year Review in Franklin County, Ohio with Most Recent Contender Phenazolam	Jennifer Hobbs
3:00-3:12 pm	S-18	High Behind Bars: A Retrospective Study of Drugs Detected in Miami-Dade Correctional Facilities	Marissa Finkelstein
3:12-3:24 pm	S-19	A PCP overdose death in Northern Virginia: A Case Study	Courtney Wardwell
3:30-4:00 PM: AFTERNOON BREAK			

WEDNESDAY, OCTOBER 29, 2025 MODERATORS: Robert Kronstrand & Cole Pajunen TOPIC: Best Practice			
TIME	**#**	**TITLE**	**PRESENTER**
4:00-4:20 pm	S-20	2025 Update on Standards Development Activities in Forensic Toxicology	Marc LeBeau
4:20-4:40 pm	S-21	Laboratory Accreditation in Forensic Toxicology: A Different Perspective	Robert Middleberg
4:40-5:00 pm	S-22	Training That Works: Feedback-Driven Refinement of a Comprehensive QTOF Training Program	Kaitlyn Palmquist-Orlando

THURSDAY, OCTOBER 30, 2025 MODERATORS: Mandi Mohr & Kristin Kahl TOPIC: Kratom Special Session			
TIME	**#**	**TITLE**	**PRESENTER**
8:00-8:20 am	S-25	The pharmacology of kratom, patterns of use, and growing diversity of kratom products in the US: implications for forensic toxicology investigations	Oliver Grundmann
8:20-8:46 am	S-26	Safety and Tolerability of Mitragyna speciosa MitraPlus™ (Kratom Extract) in Human Volunteers in the KAPTURE study following Controlled Administration of Single and 15 Multiple Daily Extract Doses	Marilyn Huestis
8:46-9:06 am	S-27	From Leaf to Lab: The Challenges of Kratom-related Deaths	Justin Brower
9:06-9:18 am	S-28	7-Hydroxy Mitragynine and Related Kratom Alkaloids in Forensic Casework	Alex Krotulski
9:18-9:33 am	S-29	Kratom Prevalence in Dallas County from 2018 to 2024	Sara Dempsey
9:33-9:48 am	S-30	The role of mitragynine versus other drugs in kratom related deaths	Robert Kronstrand
9:48-10:00 am	S-31	The Kratom Footprint: 2024 Postmortem Mitragynine Trends in Travis County, TX	Christina Smith
10:00-10:30 AM: MORNING BREAK			

THURSDAY, OCTOBER 30, 2025 MODERATORS: Anisha Paul & Stephanie Olofson TOPIC: Drugs & Driving Special Session			
TIME	**#**	**TITLE**	**PRESENTER**
10:30-10:42 am	S-32	Evaluating Abnormally High Methamphetamine Concentrations and Metabolite Ratios In Driving Under the Influence of Drugs Cases	Allen Mello
10:42-10:54 am	S-33	N-2-O(h) No! The Rise of Nitrous Oxide in DUID Casework and Best Practice Recommendations	Kari Midthun
10:54-11:06 am	S-34	One Driver and Three THC Cases Supporting the Three-Legged Stool Approach to Impairment	Stephanie Olofson
11:06-11:18 am	S-35	Rapid Sample Preparation and Screening of Gabapentin in Oral Fluid Using LDTD-MS/MS	Megane Moreau
11:18-11:30 am	S-36	Three-Year Review of Methamphetamine Findings in Suspected Impaired Driving Cases in Ontario, Canada	Zachary Currie
11:30-11:42 am	S-37	Hidden Dangers: Potential Unintended Fentanyl Exposure in DUID Drivers	Edward Zumaeta
12:00-2:00 PM: LUNCH			

THURSDAY, OCTOBER 30, 2025 MODERATORS: Marilyn Huestis & Michael Fagiola TOPIC: Awards			
TIME	**#**	**TITLE**	**PRESENTER**
2:00-2:12 pm	S-39	Determination of Synthetic Cannabinoids in MGG-Stained and Unstained Blood Smears: Innovations in Modern Toxicology	Karolina Nowak
2:12-2:24 pm	S-40	Synthetic Cannabinoid Quantification on Infused Paper from Prisons by Liquid Chromatography Tandem Mass Spectrometry	Nickolas Khorozov
2:24-2:36 pm	S-41	Defining Identification Criteria for Routine LC-QTOF-MS Targeted Analysis	Maria Sarkisian
2:36-2:48 pm	S-42	Analysis of Cannabinoids and Semi-synthetic Cannabinoids in Authentic Breastmilk Samples by Liquid Chromatography-Tandem Mass Spectrometry	Marco Ballotari
2:48-3:00 pm	S-43	Fast & Forensic: rapid detection & quantitation of 30 + emerging Novel Psychoactive Substances in hair	Seokjin Hwang
3:00-3:30 PM: AFTERNOON BREAK			

FRIDAY, OCTOBER 31, 2025 MODERATORS: Kaitlyn Palmquist-Orlando & Edward Zumaeta TOPIC: Various Topics			
TIME	**#**	**TITLE**	**PRESENTER**
8:00-8:12 am	S-44	Community-Driven Surveillance of Emerging Drug Trends: 2024 Findings from Project EAGLE FANG in St. Louis County	Amber Fregalette
8:12-8:24 am	S-45	Is Hydrolysis Necessary in Urine Novel Psychoactive Substance Testing?	Alicia Bland
8:24-8:36 am	S-46	Increasing the Ethanol Stop Limit Testing in Montana: Lessons Learned 1 Year Later	April Sheets
8:36-8:48 am	S-47	A Tale of Two Propafenones: Utilizing a Fit-For-Purpose Method Validation and Method of Standard Addition for the Quantitative Analysis of Infrequently Encountered Analytes	Elisa Shoff
8:48-9:00 am	S-48	Detection of cannabinoids in breath after cannabis-infused edible consumption	Jennifer Berry

FRIDAY, OCTOBER 31, 2025 MODERATORS: Kaitlyn Palmquist-Orlando & Edward Zumaeta TOPIC: Various Topics			
TIME	**#**	**TITLE**	**PRESENTER**
9:00-9:12 am	S-49	Stability of THC Concentrations in Breath Samples from Regular Cannabis Users after Overnight Abstinence from Cannabis Use	Jennifer Berry
9:12-9:24 am	S-50	Assessing Real-Time Impairment Using the DRUID® App Following Alcohol and Cannabis Use in a Controlled Study.	Sumandeep Rana
9:24-9:36 am	S-51	Rocky Mountain "High" THC concentrations in suspected impaired drivers in Colorado – a one-year analysis	Vanessa Beall
9:36-9:48 am	S-52	Examination of High Alcohol Concentrations in Drug-Facilitated Sexual Assaults	Jessica Ayala
9:48-10:00 am	S-53	Assay Effective in Identifying Synthetic Urine Products - Potential Solution for Workplace and Clinical Drug Testing	Svante Vikingsson
10:00-10:30 AM: MORNING BREAK			

FRIDAY, OCTOBER 31, 2025 MODERATORS: Tyson Baird & Kayla Neuman TOPIC: NPS & Postmortem			
TIME	**#**	**TITLE**	**PRESENTER**
10:30-10:42 am	S-54	Investigating the In Vitro Metabolism of Several Brorphine-Based Novel Synthetic Opioids Using Human Liver Microsomes	Kirk Hering
10:42-10:54 am	S-55	Case Series Involving N-Isopropyl Butylone: A Novel Synthetic Cathinone Implicated in Fatalities	Sara Walton
10:54-11:06 am	S-56	Extended Release, Extended Risk: A Fatal Case of Bupropion and Trazodone Toxicity	M. Elizabeth Zaney
11:06-11:18 am	S-57	Quantitative Analysis and Postmortem Redistribution Evaluation of Select NPS Benzodiazepines in Postmortem Casework	Elisa Shoff
11:18-11:30 am	S-58	Gamma Hydroxybutyrate: What Does the Concentration Mean? Review of Ante-mortem and Postmortem Casework from 2020 – 2025	Laureen Marinetti
11:30-11:42 am	S-59	When Routine Toxicology is Negative: The Critical Role of Seized Drug Analysis in a Medicolegal Death Investigation Involving Vaping	Jeffrey Walterscheid
11:42-11:54 am	S-60	The Cost of Clarity: Death After Participation in a Heart Protocol Ceremony Involving MDMA and Ketamine	Diane Moore

WEDNESDAY, OCTOBER 29, 2025 POSTER SESSION 12-2PM MODERATORS: Celeste Wareing & Laerissa Reveil		
#	**TITLE**	**PRESENTER**
P-001	Non-Invasive Detection of Psychoactive Substances in Breath Samples: Development of a Breath Collection Device	Aslı Atasoy Aydin
P-002	Forensic Toxicology Data-Independent Analysis Screening Using Xevo™ MRT Mass Spectrometer Routine Parts-per-Billion (ppb) Mass Accuracy	Nayan Mistry
P-003	Detection of Tier I and Tier II Drugs in Oral Fluid Samples Collected in Missouri DUI Cases	Matthew Levitas
P-004	Leveraging QTRAP technology for ultra-sensitive quantitation of 11-nor-9-carboxy-THC (THC-COOH) in hair	Ray Giska
P-005	Assessing the Long-Term Stability of Synthetic Cannabinoids in Human Blood by LC-QQQ-MS	Katya Beltran
P-006	Distribution of Fentanyl in Postmortem Brain Specimens	Grayce Behnke
P-007	Double Designers: Detection of Bromazolam and Metonitazene in Postmortem Casework	Mackenzie Liebl
P-008	Increasing Concentrations of Fentanyl in Driving Cases in Nashville TN, a Retrospective Study from 2015-2024	Lisa Branch
P-009	Extraction of U.S. Department of Transportation Drug Testing Panel from Oral Fluid Using SPE Columns of Multiple Particle Sizes	Luette Muir
P-010	Bridging the Gap Between Biological Variability and Analytical Precision with Synthetic Matrices	Kelly Cheshire

WEDNESDAY, OCTOBER 29, 2025 POSTER SESSION 12-2PM MODERATORS: Celeste Wareing & Laerissa Reveil		
#	**TITLE**	**PRESENTER**
P-011	Development of an Analytical Method for Targeted Screening of Eutylone, N,N-Dimethylpentylone and Pentylone in Dried Blood Spots using Liquid Chromatography-Triple Quadrupole Mass Spectrometry (LC-MS/MS)	Julia Hintermeister
P-012	Comparison of Thermally Modified Polar C18 and Superficially Porous Biphenyl LC Columns for Confirmation of Prescribed Drugs and Metabolites by LC-MS/MS	Stephanie Marin
P-013	Hydrolysis of Drug Glucuronides by β-Glucuronidase is Dependent on Both pH and Type of Sugar Linkage to Drug Aglycones	Anusha Chaparala
P-014	Psilacetin's Stability Challenge: Understanding and Enhancing Its Detection in Blood	Christopher McGrowder
P-015	Evaluation of the Neogen® Gabapentin Forensic ELISA for human matrices	Tina German
P-016	Comparative Evaluation of Clean Screen® BNZ and Alternative Methods for the Extraction of Prescription and Designer Benzodiazepines	Luette Muir
P-017	Deaths Involving the Inhalation of Chloroethane	Chelsea VanDenBurg

WEDNESDAY, OCTOBER 29, 2025 POSTER SESSION 12-2PM MODERATORS: Celeste Wareing & Laerissa Reveil		
#	**TITLE**	**PRESENTER**
P-018	Sensitive quantitation of nine nitazene analogs and brorphine in dried blood spots	Pierre Negri
P-019	Tissue Distribution of THC and Its Metabolites in Rats Following Exposure to a Vaped THC	Pierce Dolan
P-020	Strategies for Mitigating the Effects of High Gabapentin Concentrations in Urine Specimens	Haley Berkland
P-022	Quantification of Buprenorphine in Dried Blood Spots	Tia Freeman
P-023	Δ8 vs. Δ9-THC: Detection, Quantification, and Forensic Implications in Human-Performance Toxicology	Faith Pilacik
P-024	A Validated LC-MS/MS Method for Addressing Interferences of Norfentanyl by Synthetic Fentanyls in Urine	David Barajas
P-025	Copper State Biosurveillance: Analyte Panel Extension for Detecting Drugs of Abuse in Urine by LC-HRAMS Using Residual Hospital Samples	Lucas Erickson
P-026	Conversion of an existing norfentanyl immunoassay to a fentanyl immunoassay	Aaron Vollrath

WEDNESDAY, OCTOBER 29, 2025 POSTER SESSION 12-2PM MODERATORS: Celeste Wareing & Laerissa Reveil		
#	**TITLE**	**PRESENTER**
P-027	Disappearing Delta-8 Carboxy-Tetrahydrocannabinol in Urine During Dilute and Shoot Quantification with Liquid Chromatography-Tandem Mass Spectrometry	Yubo Chai
P-028	Optimizing hair decontamination for cocaine: evaluating solvents, wash frequency, and agitation methods	Davina Jetoo
P-029	Quantitative analysis of Δ8- and Δ9-THC and their main oxidative metabolites in urine by GC/MS and GC-MS/MS	Maiko Kusano
P-030	Performance evaluation of antibody and antigen conjugate pair for detection of xylazine and its metabolite hydroxy xylazine in lateral flow assay	Maria Voutilainen
P-031	Can desloratadine overdose result in death?	Bogdan Tokarczyk
P-032	Trends in Tranq 2: The Emergence of Medetomidine with Xylazine in Oral Fluid Toxicology Cases in the American Great Lakes Region	Chris Thomas

WEDNESDAY, OCTOBER 29, 2025 POSTER SESSION 12-2PM MODERATORS: Celeste Wareing & Laerissa Reveil		
#	**TITLE**	**PRESENTER**
P-033	Evaluation of Phosphatidylethanol (PEth) as a Biomarker for Alcohol Use in Decomposed Postmortem Blood Samples	Carrol Nanco
P-034	Cross-reactivity assessment of a highly sensitive polyclonal antibody for the detection of multiple Nitazene analogues	Caroline Johnston
P-035	The Line Goes Up?: Examining Fentanyl Concentrations Over the Years	Stuart Kurtz
P-036	Semi-quantitative simultaneous screening of multiple drugs of abuse from a single urine sample on the new Random Access Biochip Technology Analyzer (RABTA)	Caroline Johnston
P-037	A case report involving fatal MDPiHP intoxication	Munchelou Gomonit
P-038	Concentrations of Fentanyl in Hair Collected for Court-Ordered Mandatory Drug Testing	Megan Grabenauer
P-039	Using the New Stellar Mass Spectrometer to Confirm and Quantify 31 Drugs of Abuse, Including THC, in Oral Fluid	Courtney Patterson

WEDNESDAY, OCTOBER 29, 2025 POSTER SESSION 12-2PM MODERATORS: Celeste Wareing & Laerissa Reveil		
#	**TITLE**	**PRESENTER**
P-040	Determination of Nitazenes in Postmortem Casework and their Prevalence in the State of Maryland	Michael Fagiola
P-041	Using the New Stellar Mass Spectrometer and Automated Extraction for Drugs of Abuse Testing in Whole Blood	Courtney Patterson
P-042	An Improved Buffer-Surfactant System for Acute Organophosphate Poisoning Detection by Testing Human Red Blood Cell Acetylcholinesterase Activity	Pucheng Ke
P-043	Evaluation of Hydrogen Carrier Gas for the Quantitative Analysis of Volatile Compounds in Biological Fluids using Headspace Dual Column Gas Chromatography with Flame Ionization Detection	Rebecca Wagner
P-044	Launch of Resource Directory for Knowledge Transfer Between Scientific Instrument Users	Rebecca Wagner
P-045	Rapid EtG/EtS Analysis in Normal and Disease State Urine by LC-MS/MS	Jamie York
P-046	Unmasking Isobutylene: A Rare Toxicity from a Widely Used Industrial Gas	Katie Blazek

WEDNESDAY, OCTOBER 29, 2025 POSTER SESSION 12-2PM MODERATORS: Celeste Wareing & Laerissa Reveil		
#	**TITLE**	**PRESENTER**
P-047	The Cheesehead Truth: A 5 year review of Wisconsin impaired driving cases	Kristina Martinet
P-048	Cannabinoid Profiling Across Toxicology Samples in Adolescents and Young Adults by Route of Administration and in Relation to Depression Symptoms	Natasha Wade
P-049	Enhanced Sample Preparation in Urine Drug Testing: A Comparative Study of Enzymatic Hydrolysis Methods for Pain Management Drugs with Focus on Morphine and Its Glucuronide Metabolites	Lakshminiranjan Vanimireddy
P-050	Blood Alcohol and Inhalants of Abuse Analysis Using Gas Chromatography Optimized with Computer Software.	Ramkumar Dhandapani
P-051	Differentiation of Fentanyl and Its Isomeric Analogs Ortho/ meta/para-methylacetylfentanyls for Federal Workplace Drug Testing by LC-MS/MS	David Barajas
P-052	Determining the Accuracy of the N-Propanol Peak Area Statement in Postmortem Blood Volatiles Casework	Brianna Lehr

WEDNESDAY, OCTOBER 29, 2025 POSTER SESSION 12-2PM MODERATORS: Celeste Wareing & Laerissa Reveil		
#	**TITLE**	**PRESENTER**
P-053	Validated Oral Fluid and Urine Toxicology Screening RUO Workflows With DART-MSMS	Francois Espourteille
P-054	Some Old Drugs Never Go Out of Style: Quantitation of Phencyclidine (PCP) by Gas Chromatography with Mass Spectrometry (GC-MS) in Postmortem Toxicology Cases (2023-2025)	Kevin Shanks
P-055	The Important Contribution of Glucuronide Metabolite Hydrolysis to Detection and Quantitation in Urine Drug Testing by LC-MS/MS	Thomas Rosano
P-056	Homogeneous Enzyme Immunoassays for Fentanyl, Norfentanyl and Xylazine	Rajendra Singh
P-057	Detection and validation of PCP, ketamine, analogs and metabolites in blood using LC-MS/MS	Andrea Bernal
P-058	Sting Operation: Identification of Grayanotoxins in “Mad Honey” via Direct Analysis in Real Time Time-of-Flight Mass Spectrometry	Jessemia Meekins
P-059	From Bud to Puff: Characterizing Cannabinoids in E-Cigarette Emissions	Jessemia Meekins

THURSDAY, OCTOBER 30, 2025 POSTER SESSION 12-2PM MODERATORS: Kristina Martinet & Courtney Wardwell		
#	**TITLE**	**PRESENTER**
P-060	The Prevalence of Designer Benzodiazepines in Tarrant County, TX	Connie Lewis
P-061	Accelerating Health Risk Assessment of PFAS Exposure in 10 seconds: A Toxicological Approach Using LDTD-MS/MS	Sarah Demers
P-062	Assessing a New Recombinant β-glucuronidase Enzyme for the Analysis of Cannabinoids in Urine	Marco Ballotari
P-063	The Reemergence of Carfentanil in the State of Florida: A Recent Case Series	Marco Ballotari
P-064	The Science of Connection: Analyzing the SOFT Professional Mentoring Program's Journey	Ashley Johnson
P-065	CANTERA™: AI-Driven Enzyme Development for Enhanced Opioid Metabolite Hydrolysis in Toxicology	Isabel Fredes
P-066	Relative Concentration Distributions of Clonazepam and 7-Aminoclonazepam in a Large-Scale Oral Fluid Study	Tiara Evans
P-067	Long-term Stability of Isotonitazene and Protonitazene in Whole Blood	Amanda Pacana

THURSDAY, OCTOBER 30, 2025 POSTER SESSION 12-2PM MODERATORS: Kristina Martinet & Courtney Wardwell		
#	**TITLE**	**PRESENTER**
P-068	Cross-Reactivity of 53 Cannabinoid Analogs and Metabolites in Urine Using Enzyme-Linked Immunosorbent Assay (ELISA) and Homogenous Enzyme Immunoassay (HEIA) Carboxylic Acid and Immunalysis Synthetic Cannabinoid Kits	Taylor Yates
P-069	The Benefits of Centrifugation on Oral Fluid Analysis: Examining Volume and Analyte Recovery	Samantha Herbick
P-070	Rapid Differentiation of Δ^9-Tetrahydrocannabinol and Δ^8-Tetrahydrocannabinol in Oral Fluid Samples Using LC-MS/MS and Silver Ion Complexes	Corey Widman
P-071	GHB Detection in a Drug-Facilitated Crime – A Case Report	Chelsea VanDenBurg
P-072	Comprehensive Drug Analysis in Hair Samples: Extraction & UHPLC-MS/MS Quantification	Jonathan Danaceau
P-073	Post-Mortem Drug Screening of 364 Drugs Using a Combination of LC-QQQ and LC-QTOF with Positive and Negative mode Electrospray Ionization	Glen Shoemaker

THURSDAY, OCTOBER 30, 2025 POSTER SESSION 12-2PM MODERATORS: Kristina Martinet & Courtney Wardwell		
#	**TITLE**	**PRESENTER**
P-074	Prevalence of Stimulant Drugs and Benzodiazepines in Sexual Assault Casework in Dallas County from 2018 - 2023	Lindsay Glicksberg
P-075	Long-Term Stability of Tricyclic Antidepressants in Human Urine	Michael Baldwin
P-076	Oral Fluid Collection Device Stability Assessment of Twenty-Two Analytes in Human Saliva	Michael Baldwin
P-077	Observed Prevalence of Delta-8 and Delta-9-carboxy-THC by Age in the Kansas City Area	Savannah Bullinger
P-078	Method Optimization and Validation for the Detection of Single Doses of Diphenhydramine in Hair Specimens from a Controlled Dosing Study	Kaylyn Keith
P-079	Effective Communication Can Transform a Toxic Result	Laura Friederich
P-080	Forensic Detection of Psychoactive Drugs in Environmental Matrices: Method Development, Degradation study, and Chiral Insights	Ali Alawi
P-081	Screening of a Comprehensive Panel of 29 Drugs in Human Saliva Using LC-MS/MS and the Quantisal® Collection Device	Kavinda DeSilva

THURSDAY, OCTOBER 30, 2025 POSTER SESSION 12-2PM MODERATORS: Kristina Martinet & Courtney Wardwell		
#	**TITLE**	**PRESENTER**
P-082	When Things Have to go Fast – Screening Drug Paraphernalia using DART-HRMS	Jürgen Kempf
P-083	Advanced Analysis of Controlled Substances Using DART-TIMS-QTOF Mass Spectrometry	Artem Filipenko
P-084	Drug Screen Suite: A Simplified but Comprehensive Solution for Toxicological Routine Screening Using Liquid Chromatography - High Resolution Mass Spectrometry	Birgit Schneider
P-085	One Month Snapshot of the Prevalence of Anti-Epileptic Drugs in Central Virginia	Delisa Dalgleish
P-086	Unrealized Gains: Toxicology HPLC-MS/MS Method Optimization Using a Multi-factorial Approach in Oral Fluid and Urine Matrices	Phillip Gibbs
P-087	Evaluation of the Performance of the ARK™ Fentanyl II Assay	Amanda Jenkins
P-088	Detection Rates of Fentanyl in Oral Fluid and Urine Specimens in the North Region of the United States	Bing Pang
P-089	Repurposing Validated Forensic Toxicology Methods for Quantitating Gamma-Hydroxybutyrate (GHB) in Fermented Alcoholic Beverages	Jason Truskowski

THURSDAY, OCTOBER 30, 2025 POSTER SESSION 12-2PM MODERATORS: Kristina Martinet & Courtney Wardwell		
#	**TITLE**	**PRESENTER**
P-090	When Xanax Isn't Xanax: A Fatal Benzodiazepine Toxicity Involving Bromazolam and Desalkylgidazepam from Suspected Counterfeit Alprazolam	Elizabeth Taylor
P-091	Prevalence of medetomidine, xylazine, and their metabolites in an addiction medicine setting.	William Slade
P-092	Determining the Detectability and Potential Target Epitope of 212 Fentanyl Analogs and Synthetic Opioids using the Abbott iSCREEN™ Urine Test DX Drug Screen Tox Cup	Carl Wolf
P-093	The Potential Forensic Implications of Latent Alcohol Exposure by Blood Donors	Carl Wolf
P-094	One Extraction, Sixty-Four Drugs: LC/MS/MS Oral Fluid Method Validation per NSC Drug Testing and ANSI/ASB 036 Standards	Emily Bouso Raley
P-095	Evaluation of Blood and Oral Fluid Collection Methods: A Pilot Study of Over 100 Samples	Deneshia Williams
P-096	Validation of National Safety Council Tier 1 Scope of Analysis in Oral Fluid using Immunalysis ELISA kits on a Semi-Automated Tecan Freedom EVO 75	Steven Fleming
P-097	A novel DESI MS-based screening method for ultra-rapid detection of drugs metabolites in urine.	Patrice Ohouo

THURSDAY, OCTOBER 30, 2025 POSTER SESSION 12-2PM MODERATORS: Kristina Martinet & Courtney Wardwell		
#	**TITLE**	**PRESENTER**
P-098	Oops, I Injected Too Much! Smart Solutions for High Analyte Concentrations and Carryover in LC-MS Monitoring	Courtney Patterson
P-099	Autopsy and toxicologic approach to suspected laundry detergent exposure.	Ifran Chaudhry
P-100	Simultaneous identification and quantitation of NPS in human whole blood using QTRAP technology	Holly Pagnotta
P-101	A fully customizable workflow for the resubmitting of samples using customized rules without user intervention	Adam Campbell
P-102	Multi-Phase Validation of LC/QTOF-MS as a Screening Technique for Blood and Oral Fluid	Tara Federico
P-103	Recent Cases Involving an Uncommon Synthetic Stimulant: α-PVP	Erin Spargo
P-104	Let's Test That: A Novel, Single Instrument Setup for Compliance with the ANSI/ASB 120 Standard for Impaired Driving Investigations.	Timothy Fassette
P-105	Automated Solid Phase Extraction Using MicroElution Plates for Fentanyl Analogs, Nitazenes, and Xylazine in Human Urine	John Laycock
P-106	Development and validation of an enzyme immunoassay screen and achiral/chiral LC-MS/MS confirmation methods for ketamine analysis in hair	Neil Stowe

THURSDAY, OCTOBER 30, 2025 POSTER SESSION 12-2PM MODERATORS: Kristina Martinet & Courtney Wardwell		
#	**TITLE**	**PRESENTER**
P-107	Comparative Analysis of Urine and Oral Fluid Drug Testing: A 10,554-Specimen Study from Abbott Santa Rosa	Brent Dawson
P-108	An Eight-Year Retrospective Analysis of Carfentanil Presence in Nine Michigan Counties	Jade King
P-109	BTMPS in the Illicit Opioid Supply: Emerging Trends and Analytical and Interpretive Challenges	Alex Krotulski
P-110	Detection of Ortho-Methylfentanyl in Postmortem Cases in Michigan: A Surveillance Report of an Emerging Synthetic Opioid	Ivan Padilla
P-111	Four-Year Comparison of Delta-8- and Delta-9-Tetrahydrocannabinol in DUI and Homicide Cases	Hannah Strickland
P-112	Automated Extraction and Chromatographic Separation of Δ 8, 9, 10 THC Isomers from Whole Blood	Kyle Dukes
P-113	Sample Cleanup Approach for the Analysis of A Multi-Class Drugs of Abuse Panel in Breast Milk	Kyle Dukes
P-114	LC-MS/MS Analysis of Benzodiazepines in Urine with Room Temperature Hydrolysis and One-Step Clean Up Using Beta -Gone	Andre Sukta
P-115	Enhancing Forensic Toxicology Through Post-Mortem Applications: Evaluating the Viability of Skin Cells as a Drug Testing Matrix	Hannah Schaeffer
P-116	Development of a Quantitative Method for Opioids in Blood Using LC-MS/MS and Solid Phase Extraction	Alexus Ramirez-Wiggins

S-01

The Effect of Vaporized and Oral Δ8-Tetrahydrocannabinol vs Δ9-Tetrahydrocannabinol on Pharmacokinetics and Pharmacodynamics in Healthy Adults

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Abstract

Introduction: Δ8-Tetrahydrocannabinol (Δ8-THC), a structural isomer of Δ9-tetrahydrocannabinol (Δ9-THC), has emerged as a drug of concern in the retail "hemp" market due to its abuse liability and potential to cause intoxication and impairment. While Δ8-THC is widely marketed as a "milder" alternative to Δ9-THC, empirical data on its pharmacokinetics (PK), pharmacodynamics (PD), and behavioral effects, including how it affects driving behaviors, remain limited. Preliminary evidence suggests that Δ8-THC produces psychoactive effects similar to Δ9-THC, but its potency, timecourse, magnitude of effects, and overall safety profile remain poorly defined.

Objectives: This controlled human laboratory study aims to characterize subjective, behavioral, physiological and PK effects of vaporized (sub-study 1) and oral (sub-study 2) Δ8-THC, compared to vaporized/oral Δ9-THC and placebo.

Methods: Healthy adults (sub-study 1: N=8; sub-study 2: N=7) completed four double-blind, outpatient drug administration sessions during which they self-administered Δ8-THC (30mg, 60mg), Δ9-THC (30mg), or placebo. Outcome measures were assessed at baseline and over 6 (vaporized) or 8 (oral) hours post-administration and included: self-reported drug effects, cognitive and psychomotor assessments, simulated driving performance, standardized field sobriety tests (SFSTs), and physiological outcomes. Blood, urine, and oral fluid specimens were obtained to characterize PK of Δ8-THC, Δ9-THC, and their metabolites.

Results: All active drug conditions were generally well-tolerated with no serious adverse events. Peak subjective drug effects followed a dose-dependent pattern, with 60mg Δ8-THC and 30mg Δ9-THC eliciting the highest ratings, followed by 30mg Δ8-THC and placebo. Subjective effects generally peaked between 0-1 hour for vaporized and 2-3 hours for oral administration. Both Δ8-THC and Δ9-THC impaired cognitive and psychomotor performance, peaking between 0-1 hour and 2-5 hours following vaporized and oral administration, respectively. Driving performance was impaired by all active drug conditions. Notably, vaporized Δ8-THC and Δ9-THC produced comparable impairments, while oral 30mg Δ9-THC produced greater impairment than oral Δ8-THC at either dose. Field sobriety tests were largely ineffective at detecting Δ8-THC or Δ9-THC impairment, with no observed differences between placebo and active conditions across doses for either route of administration. Lastly, both Δ9-THC and Δ8-THC doses increased heart rate relative to placebo. PK results are pending and the study is ongoing with a target sample size of 20 completers.

Discussion: Consistent with prior research, these preliminary results indicate that Δ8-THC produces acute behavioral and physiological effects that are qualitatively similar to those of Δ9-

THC. The observed limitations of standardized field sobriety tests in detecting Δ8-THC or Δ9-THC impairment highlight the need for improved detection tools. As Δ8-THC products continue to proliferate in an evolving regulatory landscape, data from controlled human studies such as this are critical to informing science-based policy and public health education.

Disclosure

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

Conflict of Interest

Substance Abuse and Mental Health Services Administration (SAMHSA) supported this work. The authors of SAMHSA who helped prepare and contribute to this abstract did so in their personal capacity. The views and opinions expressed by these authors are their own and do not necessarily represent the views, opinions, or policies of the Substance Abuse and Mental Health Administration, the Department of Health and Human Services, or the United States government.

S-02

Blood Cannabinoid Findings in Human Performance and Postmortem Casework from 2024-2025: Implications for Forensic Toxicology Laboratories

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Abstract

Introduction: Numerous tetrahydrocannabinol (THC) isomers have been advertised in unregulated products over the last several years to take advantage of legal loopholes and increased consumer interest. For this reason, laboratories were compelled to incorporate Δ^8-THC and its metabolites into their analytical procedures with some laboratories expanding their specificity testing or scope to include additional THC isomers. Chromatographic resolution between the synthesis by-product $\Delta^{9,11}$-THC (exo-THC) and Δ^9-THC proved to be challenging for several laboratories; however, it remained unclear if this analyte or other potential cannabinoids of interest were present in toxicology specimens.

Objectives: This work will inform attendees about relevant cannabinoids observed in casework to assist laboratories in determining an efficient scope of testing.

Methods: Study inclusion criteria involved cases that screened positive for cannabinoids by immunoassay or LC-QTOF/MS (depending on the case type) and confirmed positive for at least one analyte. An upgraded LC-MS/MS confirmation panel validated according to ANSI/ASB 036 was used to monitor Δ^8- and Δ^9-THC, along with their respective hydroxy (11-OH-THC) and carboxy (THCCOOH) metabolites, and Δ^8- and Δ^9- forms of tetrahydrocannabivarin (THCV), 11-nor-9-carboxy-THCV (THCVCOOH), tetrahydrocannabutol (THCB), tetrahydrocannabiphorol (THCP), and THC-C8. Additionally, Δ^9-tetrahydrocannabihexol (Δ^9-THCH), hexahydrocannabinol (HHC) epimers, 11-nor-9-carboxy-HHC (HHCCOOH) epimers, $\Delta^{6a,10a}$/Δ^{10}-THCCOOH, and exo-THC were included. All parent and hydroxylated analytes had limits of detection of 1 ng/mL, while carboxylated analytes were 5 ng/mL. For the quantitative analytes, dynamic ranges were 1-50 ng/mL for Δ^8- and Δ^9-THC, 11-OH-THC, 9(*R*)-HHC, and 9(*S*)-HHC and 5-250 ng/mL for Δ^8- and Δ^9-THCCOOH. This analytical method was published in the 2025 May issue of the *Journal of Analytical Toxicology*.

Results: Out of 139 positive blood cases, the majority were human performance (n=133) and included fit-for-duty, driving under the influence, and alleged drug-facilitated sexual assault cases. Nearly one-quarter of the cases included Δ^8-THC and/or metabolites without any Δ^9-THC and/or metabolites. Both Δ^8-THC and Δ^9-THC and/or their metabolites were present in 24% of cases. Two-thirds of Δ^9-THC positive cases contained 11-OH-Δ^9-THC, whereas one-quarter of Δ^8-THC positive cases contained 11-OH-Δ^8-THC. Human performance Δ^8-THC (n=33) and Δ^9-THC (n=74) concentrations were similar with means of 6.3 ± 7.0 and 5.4 ± 5.7 ng/mL, medians of 3.3 and 3.7 ng/mL, and maximums of 25 and 29 ng/mL, respectively. Postmortem concentrations ranged from 2.4 - 7.6 ng/mL for Δ^8-THC (n=2) and 1.2 - 20 ng/mL for Δ^9-THC (n=4). 9(R)-HHCCOOH was found in 40% of cases, while 9(S)-HHCCOOH was observed in just 1% of cases. Of interest, exo-THC was not detected in any of the 139 blood samples. $\Delta^{6a,10a}$/Δ^{10}-THCCOOH was present in 32% of specimens and was always in combination with Δ^9-THCCOOH. HHC parent epimers appeared in nine blood cases, with only one case containing 9(*R*)- and 9(*S*)-HHC without Δ^8- and Δ^9-THC

or metabolites. Δ^9-THCP was the most common homolog detected (n=10), and was the only analyte detected in two cases. Meanwhile, 9(*R*)-HHCP was detected in three cases but was never observed without Δ^8-THC. Δ^8- and Δ^9-THCV, THCB, THC-C8, Δ^9-THCH, and 9(*S*)-HHCP were not detected in any blood specimens.

Discussion: Forensic toxicology laboratories are challenged with providing comprehensive testing services to their stakeholders while staying mindful of budget, turnaround times, and efficiency. However, an extensive cannabinoid panel may be cost-prohibitive and arduous for high-throughput forensic toxicology laboratories. This study found that Δ^8-THC continues to persist in forensic toxicology casework and may be detected in the absence of Δ^9-THC and metabolites. In addition, exo-THC does not appear to be an analytical confounder in blood toxicology analyses since it was not found in specimens, at least at this time. Based on the findings of this study, laboratories should consider adding Δ^9-THCP to their standard blood cannabinoid panels.

Disclosure

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

S-03

From Exposure to Evidence: Recent Casework Involving Cannabinoid Use and Adverse Events

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Abstract

Introduction: The unregulated cannabis marketplace has proliferated in recent years, largely due to regulatory gaps in the 2018 Agricultural Improvement Act. Many unregulated products have been identified as containing unlabeled ingredients, or ingredients listed with inaccurate concentrations. Additionally, many products contain synthetic cannabinoids, such as delta-8 tetrahydrocannabinol (Δ8-THC) and delta-9 tetrahydrocannabiphorol (Δ9-THCP), which have little to no known health or safety data, yet these products frequently include recommended dosing regimens. These manufacturing practices and quality assurance gaps put consumers at risk for adverse events.

Objectives: To chemically characterize products submitted as part of casework in order to determine pharmacologically active constituents.

Methods: Case one included two cannabinoid-based vaping products that were submitted after an individual was hospitalized for a few days following their use. A family member reported the individual felt like they were in a “dreamlike state”, “was unable to speak at times”, and went “catatonic for periods of time”. The first product, a Modus Breezy Blend “Pina Colada Ice” vape, was labeled to contain “CB9-A, Pegasus Delta 8, and THC-P”. The second product, a Magnolia Hemp “Rainbow Runts” vape was labeled to contain “THC + CBD”.

Case two included three vaping products, two oral tinctures, and one package of gummies. The products were submitted by a woman stating her husband has been missing since April 5th, 2025. On last contact, she noted “erratic behaviors” with delusional ideations, reporting he kept referring to “the flood”. She reported her husband had a severe opiate addiction, and was concerned he was using products “laced with an opiate”. The three vapes were all Geek THCX products of varying flavors, labeled as containing “D9 + THC-P + HTE” or “THC-A melted diamonds”. One tincture, Jar Co. Hash Rosin “peppermint”, was labeled to contain THC. The other tincture, Mend “balance 1:1”, was labeled to contain “a 1:1 ratio of CBD:THC”. The final product, Cake “Strawberry Sour Diesel” gummies, were labeled as containing “Delta 9 + THC-A”.

Case three included one vaping product used to alleviate back pain. Individual reported that following increased use, started noticing hallucinations and for days following use they “didn’t feel quite the same”. The product, a Super Chill vape cart, did not have any label nor associated packaging.

Upon receipt, products were analyzed using a previously published untargeted screening method utilizing a Shimadzu QP-2020 gas chromatograph mass spectrometer (GC-MS). Cannabinoids found in the products were quantitated using a previously published method utilizing a Shimadzu LCMS-8050 liquid chromatograph tandem mass spectrometer (LC-MS/MS). A second method using LC-MS/MS was employed for further screening of morphine, amphetamine, codeine, methamphetamine, norfentanyl, buprenorphine, cocaine, fentanyl, and methadone.

Results: The major cannabinoids identified in each product are as follows: Modus, D8-THC (34%), D9-THC (3.1%), D9-THCP (1.2%), D-4(8)-iso-THC (TBD); Magnolia Hemp, CBD (14.9%), D8-THC (13.3%), D9-THC (1.9%); Geek Blackberry Kush, D8-THC (28.8%), D9-THC (5.0%), D9-THCP (4.9%), D-4(8)-iso-THC (TBD); Geek Wedding Cake, D8-THC (26.0%), D9-THC (3.9%), D9-THCP (3.9%), D-4(8)-iso-THC (TBD); Geek Granddaddy Purple (qualitative only due to minimal sample), D8-THC, D9-THC, D9-THCP; Jar Co., D9-THC (0.8%); Mend, CBD (0.4%), D9-THC (0.4%); Cake, no active compounds; Super Chill, CBD (25.3%), MDMB-4en-PINACA (TBD). Quantitation of delta-4(8)-iso-THC and MDMB-4en-PINACA is forthcoming. No opioids or other psychoactive ingredients were identified.

Discussion: Analysis demonstrates the ongoing issue with quality assurance and labeling practices in the unregulated market. Incorrectly labeled concentrations, and inclusion of unlisted, pharmacologically active compounds, may be the cause of untoward effects. Synthetic cannabinoids, including those federally scheduled, continue to be identified in consumer products.

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Disclosure

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

S-04

Lipid Lifeboats: Capturing Fentanyl with Bioengineered Liposomes

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Abstract

Introduction: Fentanyl and related synthetic opioid agonists, which produce lethal respiratory depression, are the leading causes of drug overdose even with increased access to opioid overdose reversal agents such as naloxone. Despite naloxone's ability to rapidly restore compromised respiration, multiple doses are often required due to naloxone's shorter half-life compared to fentanyl. Liposomes, already used in many therapeutic drug formulations, are nanocarriers comprising lipophilic phospholipid bilayer membranes and aqueous cores. Liposomes have been proposed as a novel treatment strategy which will leverage fentanyl's physiochemical properties by sequestering unbound fentanyl to complement existing opioid overdose reversal practices, resulting in a sustainable reversal of fentanyl-depressed respiration that mitigates the risk of renarcotization.

Objectives: To evaluate the efficacy of bioengineered liposomes for sequestering fentanyl in phosphate buffered plasma (PBS) and human serum samples.

Methods: Liposomes were manufactured with a 1:1 ratio of dimyristoyl phosphatidylcholine (DMPC): 1,2-dioleoyl-sn-glycero-3-[phospho-rac-(1-glycerol)] (DOPG) phospholipids. An analytical method for identification and quantitation of fentanyl was developed using a Shimadzu LCMS-8050 liquid chromatograph with tandem mass spectrometer (LC-MS/MS).

Two sets of calibrators (0.1 – 150 ng/mL fentanyl; n=1 each), quality controls (0.3, 50, and 100 ng/mL fentanyl; n=3 each), blanks (n=3), and double blanks (n=3) were prepared in water to a volume of 100 µL. 900 µL of either PBS or fentanyl-free authentic human serum was then added to half of each sample set. Samples were prepared as 25, 50, and 100 ng/mL fentanyl (n=6 for each concentration and matrix) in water to a volume of 100 µL. 100 µL of water or liposomes were then added to half of each matrix/concentration. The total liposome loading for the prepared samples was 1.44 mg lipid/mL. Calibrators, QCs, and samples with and without liposomes were vortexed for 15 minutes, then 800 µL of either PBS or serum was added to samples. All calibrators, QCs, and samples were then centrifuged for 15 minutes, transferred to a syringe with a 0.2 µm filter, collected into an autosampler vial containing internal standard, and analyzed using the LC-MS/MS method.

Results: Analytical results demonstrate that regardless of starting fentanyl concentration, liposomes were effective at binding approximately 80% of the fentanyl in PBS samples. In serum, liposomes bound approximately 50% of fentanyl, again regardless of starting concentration.

Discussion: The evaluated liposome formulations were effective in sequestering a portion of the fentanyl. Considering fentanyl also is highly protein bound (~80-85%), the free fentanyl in blood following liposome administration is expected to be ~11% of the total dose administered. Fentanyl

sequestering in liposomes should drive tissue bound fentanyl to diffuse out into the blood and away from active receptors. While future in vitro work will investigate other engineered liposome formulations, the results are encouraging for use of liposomes as a novel therapeutic to combat fentanyl overdoses. In vivo animal studies are planned to ensure outcomes are consistent with the presented in vitro results.

Funding: This project was supported in part by the National Institutes of Health [P30DA033934].

Disclosure

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

S-05

Determination of Neurotransmitter Levels in Rat Brain Following Opioid-Sedative Exposure Using Liquid-Chromatography Quadrupole-Time-Of-Flight Mass Spectrometry

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Abstract

Introduction: Neurotransmitters (NTs) play an essential role in the function of our nervous system with memory, learning, and cognition. Exposure to drugs used clinically or illicitly like opioids and sedatives can cause delirium, a condition from which cognitive dysfunction occurs. This dysfunction is thought to result from NT imbalance. Behaviorally, delirium is exhibited by observable changes in attention, thinking, memory, psychomotor function, perception, and mood. Untreated delirium contributes to increased morbidity and mortality rates and can lead to reduced quality of life and long-term cognitive impairment. Clinical diagnosis of delirium relies on behavioral assessments, which are subjective and have low success rates in delirium detection. Quantification of changes in NT levels would provide demonstrative evidence for the effect of sedation in the development of delirium.

Objectives: To validate a bioanalytical method to measure dopamine (DOP), serotonin (SERT), glutamic acid (GLU), gamma-aminobutyric acid (GABA), gamma-hydroxybutyric acid (GHB), and acetylcholine (ACT) levels in rat plasma and brain.

Methods: Five NTs, in positive mode, and GHB, in negative mode, were optimized and chromatographically separated using liquid chromatography quadrupole-time-of-flight mass spectrometry. The standard curve of 10-5000 ng/mL in 90:10 ACN:H_2O and quality controls (QCs), prepared in diluted, pooled rat plasma, were extracted using a protein precipitation method with ice-cold 50:50 H_2O:MeOH + 0.5% formic acid and evaluated for accuracy, precision, carryover, and stability over four days following the Food and Drug Administration M10 Bioanalytical Method Validation and Study Sample Analysis Guidance for Industry. The method was also partially validated over three days for accuracy and precision of QCs prepared in diluted, pooled rat brains.

The method was applied to measure NTs from rats in a delirium model. Male and female Sprague-Dawley rats were repeatedly treated with saline (n=24), lipopolysaccharide-induced inflammation (LPS; n=20), morphine- (2-4 mg/kg/dose) and midazolam- (5-9 mg/kg/dose) induced sedation (MOR/MDZ; n=17), or combined LPS and MOR/MDZ (n=15) over 5 days. Rat brains were halved and homogenized in HALT protease inhibitor cocktail. The supernatant was aliquoted and extracted for NTs.

Results: Across all six analytes, the limit of detection ranged from 5-100 ng/mL and the lower limit of quantitation ranged from 10-250 ng/mL. Within-run accuracy and precision of QCs in plasma (n=6 per level) across all analytes were within ±14% and 13%, respectively. Between-run accuracy and precision were within ±6% and 9%, respectively. Carryover was not observed. The accuracy and precision of QCs for short-term and freeze-thaw stability were within ±9% and 10%, respectively. The accuracy and precision of QCs prepared in rat plasma and brain were acceptable under validation guidelines. For ACT, SERT, DOP, and GABA, there was no significant difference in levels across the treatment groups compared to vehicle (saline). A significant

difference in GHB levels in the MOR/MDZ group (p=0.0314) compared to vehicle was observed. For GLU, there was a significant increase in levels in the MOR/MDZ group (p=0.0004) and the combined LPS+MOR/MDZ group (p=0.0349) compared to vehicle.

Discussion: We successfully validated two methods for the analysis of NTs in rat plasma and brain. These data suggest that inflammation nor sedation significantly affect the levels of ACT, SERT, DOP, and GABA. Sedation significantly increased GHB and GLU levels, and an additive effect of inflammation and sedation also increased GLU levels. However, inflammation alone did not impact GHB or GLU levels. NTs may be a promising biomarker for delirium caused by sedation.

Funding: Children's Hospital Research Institute Collaborative Award; Children's Hospital Foundation Brain Injury Program

Disclosure

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

S-06

Translating UPLC-QTOF Screening from Research to Routine: Validation, Accreditation, and Implementation for Systematic Toxicological Analysis

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Abstract

Introduction: High-resolution mass spectrometry (HRMS) combined with non-selective sample preparation (e.g., protein precipitation) offers broad analyte coverage, making it valuable for qualitative forensic drug screening. This abstract presents the validation of a UPLC-QTOF method and its implementation in a high-throughput forensic toxicology setting, specifically within the systematic toxicological analysis (STA) workflow for blood and urine in our laboratory. The aim was to evaluate the method's reliability for compound identification and its potential to reduce the need for confirmatory testing in routine casework. The validation strategy and path towards ISO-17025 accreditation are also described.

Objectives: The primary objective was to determine limits of identification (LOIs) for a wide range of toxicologically relevant compounds, accounting for matrix effects and day-to-day system variation. Compounds were selected based on their occurrence in Belgian forensic casework. The 121 compounds tested spanned the following pharmacological classes: cocaine and metabolites (4), opioids (19), amphetamine-type stimulants (9), THC and metabolites (3), benzodiazepines (30), antidepressants (27), and antipsychotics (27). Additional validation parameters included precision, processed sample stability, and carry-over.

Methods: Samples were processed via protein precipitation using acetonitrile (100 µL blood/urine to which 50 µL internal standard was added, followed by 400 µL cold acetonitrile). Analyses were performed using an Acquity I-Class UPLC coupled to a Xevo G3 QTOF-MS (Waters), operating in data-independent acquisition (DIA) mode. Identification criteria (IDC) included: retention time deviation (<0.5 min), mass error (<5 ppm), signal intensity (>3,000 counts), isotope pattern match (m/z RMS <20 ppm and intensity RMS <20 %), and detection of ≥50% of expected fragment ions. Quality assurance (QA) included daily performance checks, system suitability tests, and quality control sample analysis.

LOIs were determined over five days using six different blood and urine pools. At least three concentration levels were spiked. The LOI was defined as the lowest concentration meeting all identification criteria (IDC) in all injections. Interday precision was assessed using an average matrix pool analysed over five days. The average and variation in identification criteria at the LOI level were reported. Processed sample stability was assessed on day 0, +1, +2, and +5 by comparing day 0 results with those from later days, after storage at 4°C in the autosampler.

Results: LOIs were determined for most compounds. For cocaine and its metabolites, a LOI of 2.5 ng/mL was found. For opioids, LOIs ranged from 0.5 to 10 ng/mL; for antidepressants, from 1.25 to 80 ng/mL; for antipsychotics, from 1 to 100 ng/mL; and for benzodiazepines, from 2 to 40 ng/mL. LOIs in urine were generally higher than in blood, likely due to stronger matrix effects. Although matrix effects were not directly measured given the qualitative nature of the method they were evaluated based on variability between matrix pools. Greater variation in response and other identification criteria in urine suggest stronger matrix effects compared to blood. Opioids proved

the most challenging class, with the lowest success rate in defining LOIs. 20 % of compounds in blood and 26 % of compounds in urine could not be validated due to (1) signal suppression or interference from co-spiked analytes, (2) poor ionization efficiency, or (3) chromatographic issues.

For interday precision, average values and deviations of identification criteria remained well within IDC thresholds for most compounds, indicating acceptable performance. Processed sample stability was generally good (a maximum 20% decrease, with few exceptions). No significant carry-over was observed.

Discussion: This work presents a comprehensive validation approach for UPLC-QTOF screening. The use of multiple matrix pools and staggered analysis days ensured matrix effects and interday variability were captured in the LOI determinations. The resulting LOI dataset provides a laboratory-specific performance map, aiding forensic toxicologists in deciding when confirmatory testing is necessary based on compound class, matrix, and case context. Combined with a structured QA approach, this supports reliable routine use.

This method validation strategy and accompanying QA framework have been formally accepted under ISO-17025 accreditation, providing a robust and auditable foundation for high-quality forensic toxicology.

Disclosure

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

S-07

Impact of Stop-Limit Testing Practices at the Wisconsin State Laboratory of Hygiene… and Beyond?

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Abstract

Introduction: Drug-Impaired Driving (DUID) remains a persistent public health issue across the United States. Many forensic toxicology laboratories face resource limitations that prevent comprehensive alcohol and drug testing of every specimen received. To manage these constraints, laboratories often implement stop-limit testing policies - administrative rules that cancel drug testing when blood alcohol concentration (BAC) exceeds a certain threshold. The Wisconsin State Laboratory of Hygiene's Forensic Toxicology Program (WSLH) receives over 20,000 specimens annually and, due to budgetary constraints, limited instrument capacity, and ongoing demands to decrease turnaround time, must rely on such stop-limit policies. As a result, WSLH may underreport drug positivity rates, potentially limiting the information available to stakeholders about impaired driving trends and emerging drug threats.

Objectives: This project aims to evaluate drug positivity rates and trends in specimens where drug testing was cancelled due to BAC stop-limit thresholds.

Methods: Wisconsin law enforcement agencies submitted blood samples to WSLH for toxicology testing related to operating while intoxicated (OWI) arrests. The WSLH randomly selected cases initially submitted for comprehensive testing but, following ethanol analysis, had subsequent drug testing cancelled due to a measured BAC exceeding 0.080 g/dL (at the request of the submitting agency) or 0.100 g/dL (per laboratory policy). Specimens were screened for drugs using a Liquid Chromatography-Quadrapole Time-of-Flight Mass Spectrometry (LC-QToF) method, with a scope of over 400 substances. Drug test results were evaluated for positivity by BAC group, prevalence, and trends. Specimens were de-identified prior to testing so results cannot be attributed back to the subject.

Results: A total of 2000 blood samples were analyzed, 1000 in 2024 and an additional 1000 in 2025. In 2024, 568 samples (57%) tested positive for at least one drug in addition to a BAC above 0.080g/dL. In 2025, 590 samples (59%) showed drug positivity beyond ethanol. Excluding samples positive for only drugs not commonly associated with impairment, the percentage decreases slightly to 53% in 2024 and 56% in 2025.

The most represented BAC group in the 2024 set was 0.171-0.200 g/dL while in 2025 it was 0.121-150 g/dL. The BAC group with the highest number of additional drugs detected was 0.201-0.250 g/dL in 2024 and 0.121-0.150 g/dL in 2025. The average BAC across both years was 0.184 g/dL.

The most commonly detected drug in both years was THC metabolites (11-nor-9-carboxy-THC and its glucuronide), with 771 positive specimens (38%), followed by cocaine and its most common metabolite benzoylecgonine, detected in 177 specimens (8.8%). Positivity rates for fentanyl and opioids remained at 1%.

Discussion: These results demonstrate that the stop-limit testing policies used by WSLH may contribute to underreporting of drug use in Wisconsin drivers, a trend likely present in other

jurisdictions with similar policies. The data suggest that among Wisconsin drivers operating under the influence, over half are using drugs in addition to having a prohibited blood alcohol concentration. The QToF method used is sensitive so it should be noted that not all drugs detected are associated with impairment and some may be present at pharmacologically insignificant levels. THC metabolites were the most frequently detected substances followed by cocaine and its metabolite. Cocaine and benzoylecgonine are restricted controlled substances (RCS) under Wisconsin law. RCS drugs include Schedule 1 drugs, controlled substance analogs, cocaine and its metabolites, methamphetamine, and delta-9-tetrahydrocannabiniol (at concentrations ≥1 ng/mL). Among drug-positive cases, 60% involved THC metabolites and/or RCS-classified drugs only. A limitation of this study is that delta-9-tetrahydrocannabinol was not tested for in THC metabolite positive samples due to funding restriction. Although recommended practice would entail comprehensive drug testing for all impaired driving cases, WSLH's ability to do so is limited by budgetary and resource constraints.

Conflict of Interest

Financial support for this study from Wisconsin Bureau of Transportation Safety

S-08

Impaired Driving Surveillance in Arkansas: Year One Review.

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Abstract

Introduction: As many forensic laboratories are limited in personnel, budget, and resources, stop limit testing may be implemented as standard procedure if a driver's blood alcohol concentration (BAC) is measured above a specific cutoff, usually at or above the *per se* limit, meaning drug testing will not be performed for these cases as impairment could be demonstrated with the BAC result alone. Missing data on the driving population's drug usage has made it difficult to assess the prevalence of drugged driving and the crash risk associated with an individual that has drugs or combinations of drugs in their system.

For the Arkansas State Crime Laboratory (ASCL), DWI samples with a measured BAC above the *per se* limit of .08 g% are subject to stop testing, and drug testing is not performed on those samples. To address the loss of drug use data among impaired drivers in Arkansas, the ASCL and Glen F. Baker Public Health Laboratory (GFBPHL) collaborated to begin an Impaired Driving Surveillance Project in June 2024. De-identified blood samples from suspected impaired drivers that were subject to the ASCL stop testing policy were provided to the GFBPHL for comprehensive drug screening by the Chemical Terrorism Laboratory. By providing de-identified samples, the GFBPHL could expedite sample testing and reporting, allowing for up-to-date information on a rapidly shifting drug landscape to be disseminated to the ASCL, law enforcement, policy makers, and healthcare providers.

Objectives: The objective of the Impaired Driving Surveillance Project was to determine the prevalence of polysubstance impaired driving in Arkansas and to disseminate this data to to ASCL, law enforcement, and healthcare providers so informed decisions can be made regarding countermeasure policies. The project also aimed to demonstrate a roadmap to successful and productive collaborations between state laboratories that further the mission of each's respective agency.

Methods: The ASCL provided the GFBPHL with de-identified blood samples from impaired driving casework subject to the ASCL's stop testing policy. Samples were processed by supported liquid extraction (SLE) and analyzed via a Thermo Scientific Orbitrap Exploris 480, which was used to acquire full-scan, high-resolution MS and unit-resolution MS2 data from samples. Reference materials for each compound of interest were previously analyzed to create an in-house MS2 spectral library which was used to assist in identification and confirmation of compounds. All results were reported qualitatively.

Results: Testing commenced in June 2024. Thus far, 178 of 293 (61%) blood samples tested included drugs other than alcohol. Of the 178 drug-positive samples, 86 (48%) tested positive for 2 or more drugs in addition to alcohol. Sedatives accounted for 33% of all drug detections, followed by cannabis (19%), CNS stimulants (18%), and narcotic analgesics (13%). Delta-9 THC was the most detected compound overall, with methamphetamine/amphetamine, clonazepam, cocaine, alprazolam, and fentanyl following. Polysubstance use involving multiple drug categories was prevalent, as 71 of 178 (40%) drug-positive samples contained three or more

separate drug categories (including alcohol). Five samples tested positive for five separate drug categories. The top drug category combination was alcohol/cannabis, followed by alcohol/sedatives, and alcohol/stimulants.

Discussion: The data gathered thus far support the need to eliminate stop testing policies for DWI cases and further demonstrate that polysubstance drug use is prevalent among drivers. By using stop testing policies, critical data is missing regarding drug use habits which inhibits law enforcement, healthcare professionals, and policy makers from making informed decisions on countermeasure polices and treatments. This project also demonstrated a successful collaboration between state partners to study a public safety and health issue that one department on its own cannot address sufficiently.

Disclosure

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

S-09

Alive and Somehow Behind the Wheel

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Abstract

Introduction: Driving under the influence of drugs (DUID) continues to be a safety concern throughout the country. Zolpidem and methamphetamine are prevalent in impaired driving cases and pose a danger to public health and safety. Forensic toxicologists are often asked to discuss quantitative drug concentrations in court and explain the potential significance of those values. Standardized field sobriety tests (SFSTs) are often performed at or near the time of a driving incident to evaluate a driver's level of impairment. Toxicology testing can be used to help support those results.

Case studies have reported a wide range of drug concentrations in drivers from below therapeutic to what could be considered lethal. Information gathered from case reports can help track trends and give toxicologists more information about the impairment observed in DUID cases.

Objectives: The objective of this case report series is to describe ten DUID cases with elevated blood concentrations of zolpidem (three cases) and methamphetamine (seven cases). These cases will be used to gain more insight into surprisingly high drug concentrations found in some DUID cases.

Methods: Blood from all cases underwent the laboratory's standard screening process which included the following: ethanol (if needed), an eight-panel enzyme-linked immunosorbent assay, and a qualitative general unknown drug screen. After screening, samples were prepared for confirmation and quantification using a solid-phase extraction method for basic drugs followed by analysis on one of four validated LC/MS/MS instruments. Samples were quantified on a linear seven-point whole blood calibration curve ranging from 20 - 2000 ng/mL with 1/x weighting, not forced through zero, and $r^2 \geq 0.990$. Cases initially above the linear range were re-extracted using a smaller aliquot volume.

Results: Pertinent case information with toxicological findings and SFST results for each case is presented in Table 1. The driving scenario and officer observations for each case will be described in a narrative in a manner similar to Case 1 presented below.

Case 1: An officer witnessed a driver speeding, rapidly decelerating, and abruptly stopping in the middle of an intersection. The driver then accelerated at a high speed before stopping. The officer observed droopy eyes, slurred speech, slow speech, slow and deliberate movements, and stumbling. The driver used the vehicle for balance.

Table 1: Case Information with Toxicology & SFST Results

Case	Demographics	Toxicology Results	Amount (ng/mL)	Other Findings	Amount (ng/mL)	HGN (Max 6)	One Leg Stand (Max 4)	Walk and Turn (Max 8)	VGN Observed + Not Observed -
1	32 WF	Zolpidem	3023	Ethanol Alprazolam	Negative 48	6	2	4	-
2	49 WM	Zolpidem	3460	Ethanol Bupropion Clonazepam 7-Aminoclonazepam	** 34 23 922	6	3	5	**
3	56 WF	Zolpidem	3668	Ethanol	Negative	#	***	***	***
4	30 WM	Methamphetamine	1763	Ethanol Amphetamine Alprazolam THC-COOH	Negative 190 60 19	2	2	2	-
5	28 WM	Methamphetamine	2272	Ethanol Amphetamine Tramadol	Negative 169 36	6	2	7	+
6	37 WM	Methamphetamine	2276	Ethanol Amphetamine THC THC-COOH	Negative 77 3.7 17	***	***	***	***

7	42 WM	Methamphetamine	2615	Ethanol Amphetamine Acetaminophen	** 199 Positive	#	***	***	***
8	33 WM	Methamphetamine	2961	Ethanol Amphetamine THC 11-OH-THC THC-COOH	Negative 69 5.0 1.2 47	0	0	5	-
9	32 WM	Methamphetamine	3012	Ethanol Amphetamine Bromazolam Fentanyl Norfentanyl Cocaine Benzoylecgonine	Negative 296 Positive 59 49 42 991	4	3	4	-
10	43 BM	Methamphetamine	6591	Ethanol Amphetamine	Negative 62	6	***	***	-

** Result unknown *** Not Performed # Partially Completed

Discussion: These ten cases can be used to provide more insight into extremely high methamphetamine and zolpidem concentrations that may be present in DUID cases. They provide information on the potential impact of these drugs on driving abilities and the observations and SFST results witnessed by officers. They also illustrate examples of levels above the therapeutic ranges that are often seen in impaired driving cases. The information obtained from these cases can benefit toxicologists when evaluating toxicology findings in their own laboratories.

It is also clear from the SFST results that poly-drug use, individual variation, and tolerance may play a role in the observations that officers see when conducting these tests. This also shows that it is important to consider the entire case history along with the toxicological findings, officer observations, and SFST results when interpreting a toxicology case.

Disclosure

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

S-10

Impaired Driving Cases with Diphenhydramine

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Abstract

Introduction: Diphenhydramine is a first generation antihistamine commonly used for allergic rhinitis, in cold medications, and as a sleep aid. Impairing effects similar to other depressants including sedation and impaired driving have been reported (1, 2). The prevalence of diphenhydramine identified in drug-impaired driving (DUID) investigations is consistently high. In successive surveys of DUID toxicology laboratories, diphenhydramine was in the top 15 most prevalent drugs for 30 out of 45 (67%) and 38 out of 57 (67%) in 2020 and 2024, respectively, that included diphenhydramine in their scope of testing (3, 4). Despite its high prevalence, diphenhydramine remains in Tier II of testing recommendations.

Objectives: Highlight the prevalence, evaluate the need to be included in routine testing, and profile impaired driving cases with diphenhydramine.

Methods: Urine specimens were tested with an 11 panel enzyme linked immunosorbent assay (ELISA, Dynex DSX with amphetamines, barbiturates, benzodiazepines, buprenorphine, cannabinoids, carisoprodol, cocaine metabolite, fentanyl, methamphetamines, opioids, and oxycodone kits from Neogen) and basic drug extraction with scan gas chromatography (GC) mass spectrometry (MS, Agilent 5890/5973). A volatile analysis was performed on all blood DUID cases. This was followed by a 3 panel ELISA (benzodiazepines, buprenorphine, and cannabinoids) and a liquid chromatography with high resolution tandem mass spectrometry (LC-HRMS, Thermo Scientific QExactive) screen. The validated limit of detection for diphenhydramine by the urine GC-MS and blood LC-HRMS screens were 10 and 1 ng/mL, respectively. Urine confirmation was performed by the same GC-MS test with a contemporaneous standard. The limit of quantitation and limit of detection for blood by LC-MSMS was 10 ng/mL for both, and by GC-NPD/GC-MS was 20 ng/mL and 10 ng/mL, respectively.

Results: Diphenhydramine was identified in 16 out of 212 (7.5%) urine and 13 out of 149 (8.7%) blood DUID cases from October, 2023 to May, 2025. During that period, diphenhydramine was the 6th and 7th most prevalent drug in blood and urine, respectively. The blood mean, median and range were 99, 49, and < 10 to 407 ng/mL, respectively. At least one blood case identification may have been due to post-incident medical intervention. All but one urine and one blood case were polysubstance. The most common combinations of other drugs identified in urine were benzodiazepines (38%), cannabiniods (31%), and stimulants (31%). While opioids (62%), benzodiazepines (54%), and stimulants (46%) were the top 3 in blood. Diphenhydramine was identified at 43 ng/mL in one blood case where it was the only drug identified. The driver was speeding, crashed into a tree and was observed to be disoriented after the crash.

Discussion: Diphenhydramine is widely available without a prescription and some may not view it as a risk to traffic safety. However, research has demonstrated that both acute and chronic use can lead to driving impairment (1, 2). The incidence of diphenhydramine in DUID investigations has remained high, although it was rarely identified alone. Diphenhydramine remains in Tier II of testing recommendations. Nevertheless, laboratories should consider including it in their scope of testing. Diphenhydramine may be involved as a contributing factor

in a large number of drug-impaired driving investigations.

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Keywords

Diphenhydramine, DUID, Impaired Driving

Disclosure

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

S-11

Puff, Puff, Pass [the Alcohol Drug Test?]: A Clinical Study Assessing Impacts of Vaped Ethanol on Ethanol Testing

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Abstract

Introduction: Use of an electronic cigarette (e-cig) liquid (e-liquid) containing ethanol can result in detectable breath alcohol if a wait-period is not employed. Ad-lib vaping of an e-liquid containing ethanol has been shown to elevate urinary ethyl glucuronide (EtG), though plasma alcohol remained negative. Multiple unsolicited correspondence received by the Laboratory for Forensic Toxicology Research at Virginia Commonwealth University claimed that use of an e-liquid led to positive blood, urine, or oral fluid (OF) tests for ethanol and/or an ethanol biomarker.

Objectives: To determine if ethanol or ethanol biomarkers are detectable in subjects following use of an ethanol-containing e-liquid.

Methods: A clinical study (IRB # HM20018402) using a controlled drinking (placebo or 20% ethanol beverage) and vaping (10 four-second puffs of a placebo or 20% ethanol e-liquid at 50 and 210 min post-drinking) crossover design was completed to investigate if vaping an ethanol-containing e-liquid impacted breath (n=12), blood (n=6), oral fluid (n=9), or urinary ethanol and/or ethanol biomarker concentrations. Sample (min) were collected post-drinking as follows: blood (<1, 40, 55, 200, 215); breath (10, 20, 40, 55, 90, 120, 165, 200, 215, 240, 270 min); oral fluid (<1, 5, 20 ,40, 55, 200, 215, and 240 min); urine (as available). An Intoxilyzer 900 (CMI, Inc.) was used for breath alcohol determinations. A headspace gas chromatography with dual flame ionization method was used to quantitate ethanol in blood, urine, and oral fluid samples. A liquid chromatography with tandem mass spectrometry method was used to quantitate the following ethanol biomarkers: EtG, ethyl sulfate (EtS), 5-hydroxyindolacetic acid (HIAA), 5-hydroxytryptophol (HTOL), 5-hydroxytryptophol-β-D-glucuronide (GTOL), and two phosphatidylethanol (PEth) isoforms, 16:0-18:1 and 16:0-18:2.

Results: Seven participants (5 male, 2 female) completed all four sessions of the crossover design. Ethanol was only detected (>0.01 g%) in blood, urine, breath, and oral fluid from the 20% ethanol drinking conditions. Ethanol determinations between the placebo vape and 20% ethanol vape did not differ significantly for any blood, breath, or oral fluid in the 20% ethanol drinking condition. Ethanol was not detected in any matrix from the placebo drink with 20% ethanol vape condition. EtG formation was detected in urine from the placebo drink with 20% ethanol vape condition; otherwise, no biomarkers formation was detected in any matrix collected from the placebo drink with 20% ethanol vape condition. The biomarkers detected in blood (EtS, EtG, both PEth isoforms) and OF (EtS) did not significantly increase between the active drinking with placebo vape condition and the active drinking with 20% ethanol vape condition.

Discussion: The presented results further demonstrate vaping ethanol will not elevate blood ethanol concentrations, but can lead to detectable EtG formation in urine. Urine was not normalized to creatinine and urine was not collected at pre-determined timepoints, so

interpretation is limited. Otherwise, the presented results indicate vaping ethanol did not affect the outcomes of ethanol biomarkers analyses commonly employed in clinical or forensic toxicology. The puffing profile used in this study may not adequately mimic real-world practices. Higher ethanol concentration vaping products, and more frequent, longer duration vaping practices may impact ethanol and/or ethanol biomarker determinations. The results of this study may help with interpretations in future casework.

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Disclosure

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

S-12

Analysis of Confiscated Vaping Products from Public Schools Across the Commonwealth of Virginia During the 2024-25 Academic Year

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Abstract

Introduction: In an effort to curb underage use, the United States Food and Drug Administration (FDA) has prohibited both the sale of electronic cigarettes (e-cigarettes, vapes) to persons <21 years of age and flavors other than menthol and tobacco in cartridge-based (pod-based) products. Though recent surveys report a decrease in vaping products use among middle and high school populations, of students who reported vaping, almost 9 in 10 reported using vapes with flavors other than menthol and tobacco. E-cigarettes have been adapted to vaporize drugs other than nicotine (DOTNs), most notably Δ9- and Δ8-tetrahydrocannabinol (THC) alongside other cannabinoids.

Objectives: To characterize vaping trends in the adolescent population of Virginia by evaluating vaping products and their liquids (e-liquids) confiscated from public school students.

Methods: Vaping products confiscated from Virginia public school students during the 2024-25 academic year were shipped to the Laboratory for Forensic Toxicology Research (LFTR) in Richmond, Virginia. The date confiscated, student grade level, how the student acquired the device, and symptomology information were requested. E-liquids were screened using an untargeted gas chromatography-mass spectrometry (GC-MS) method. Volatile compounds were identified and quantified using headspace gas chromatography-flame ionization detection (HS-GC-FID). Nicotine and cannabinoid concentrations were determined using liquid chromatography-tandem mass spectrometry (LC-MS/MS).

Results: Currently, 489 submissions from 16 school systems have been received by LFTR. Disposable products were most prevalent (91%), followed by pod-based (8.8%) then refillable products (0.2%). Of vaping devices with a visibly labelled flavor (n=453), 64.5% were fruit flavored, 10.8% were a dessert flavor, 23.8% included “frozen” or “ice” in the flavor name, and 8.8% were labelled as tobacco or menthol flavored. The most reported methods of acquisition (n=93) were receiving the vape from a friend (47.3%) or purchasing themself (29.0%). The majority of submissions were confiscated from students in grades 9-12 (57.3%), while 3.9% were confiscated from students in grades 6-8, and 38.9% had no available grade information. Of the e-liquids screened on GC-MS (n=161), 90.7% contained nicotine, 8.1% contained at least one cannabinoid, and 1.2% contained neither nicotine nor cannabinoids; 88.8% contained benzoic acid, 23.6% contained menthol, and 90.1% contained one or more synthetic coolants. Detected cannabinoids included Δ9-THC, Δ8-THC, cannabidiol (CBD), cannabinol (CBN), cannabicitran (CBT), cannabigerol (CBG), hexahydrocannabinol (HHC), and tetrahydrocannabivarin (THCV). Of the e-liquids quantitated for volatiles (n=166), 4.2% contained ethanol at >1%.

Discussion: These results indicate high prevalence of flavored e-liquids, synthetic coolants, nicotine salts, and cannabinoids in the vaping products confiscated from students, similar to results from 2023-24. Disposable products continue to be the most common device type. No FDA-authorized vaping products were submitted. Results demonstrate inaccurate labeling practices concerning both e-liquid contents and their concentrations. Vape use is still a problem in youth populations, with data continuing to demonstrate ease of access to unregulated products, many of which are cannabis-based. All of the cannabis-based vaping products with associated grade information were confiscated from high school students.

Funding: Virginia Foundation for Healthy Youth [8521496], National Institute of Justice [15PNIJ-23-GG-01421-COAP], National Institute on Drug Abuse [P30 DA033934], National Institute on Drug Abuse of the National Institutes of Health and the Center for Tobacco Products of the U.S. Food and Drug Administration [U54DA036105], 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).

Disclosure

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

S-13

The Emergence of the Designer Stimulants Fluorexetamine and 2-Fluoro-2oxo-PCE in Clinical Urine Specimens

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Abstract

Introduction: Novel psychoactive substances (NPS) are known as "legal highs," as they are designed to mimic the effects of recreational drugs while circumventing legality issues. These drugs began to increase in popularity in 2010, and, as of 2022, more than 1,000 different NPS have been identified. One emerging NPS class is designer stimulants, which have been reported to cause similar effects to ketamine and phencyclidine (PCP). Fluorexetamine and its isomer 2-fluoro-2-oxo-PCE (FXE) emerged onto the illicit drug market in 2022 and are structurally similar to ketamine. Reported side effects of FXE use include hyperthermia, dehydration, and cardiac issues.

Objectives: To investigate the prevalence of fluorexetamine and 2-fluoro-2-oxo-PCE positivity in clinical urine specimens.

Methods: We retrospectively analyzed results for urine specimens submitted to Quest Diagnostics for NPS testing from across the United States. The NPS panel contains 89 NPS compounds in 6 classes. Testing was performed using a semi-quantitative method that was previously validated according to ASB and Quest Diagnostics requirements. A linear calibration curve was used and cutoffs for analyte positivity ranged from 1 ng/mL to 20 ng/mL. Fluorexetamine and 2-fluoro-2-oxo-PCE coelute in the LC-MS/MS method and are reported as the analyte combination; specimens with concentrations >2 ng/mL are considered positive for these analytes. Once FXE-positive specimens were identified, data on non-NPS drug testing using CLIA-validated methods was also retrospectively assessed.

Results: From November 2023 to December 2024, 8,384 specimens were positive for at least 1 NPS. During this period, only 1 specimen was positive for FXE. From January through April 2025, 3,909 specimens were positive for at least 1 NPS. Of these specimens, 20 contained FXE, representing 0.51% of positive specimens. Most of these specimens came from the northeast: Connecticut (13 specimens), New York (5 specimens), and Massachusetts (1 specimen). One positive specimen was from West Virginia.

All FXE positive specimens were also positive for an additional 2-, 3-, or 4-NPS classes. All 20 specimens were positive for xylazine (other illicit additive class) and bromazolam, and/or its metabolite hydroxy-bromazolam (designer benzodiazepine class). Of these 20 FXE-positive specimens, 11 (55%) were positive for 3 NPS classes (other illicit compounds, designer benzodiazepines, and designer stimulants). Eight (40%) were positive for an additional (fourth) NPS class: designer fentanyl analogs including acetyl fentanyl (6 specimens), carfentanil, and norcarfentanil (1 specimen), fluorofentanyl (3 specimens), and/or isobutyryl fentanyl/ortho-methyl fentanyl (2 specimens). One (5%) was positive for an additional (fifth) NPS class: designer opioids: desethyl metonitazene, hydroxy-nitazene, and metonitazene.

In total, 4 of the 20 FXE-positive specimens (20%) were also positive for cyclohexyl methylone, another NPS in the designer stimulant class. No drugs belonging to the 6th NPS class (synthetic cannabinoids) were detected.

In addition to positivity for numerous NPS classes, all 20 specimens were positive for other drugs of abuse including fentanyl and/or norfentanyl (>0.5 ng/mL); 13 (65%) for the cocaine metabolite, benzoylecgonine (>200 ng/mL); 10 (50%) for methamphetamine (>500 ng/mL); 6 (30%) for the heroin metabolite, 6-monoacetylmorphine (>10 ng/mL); 12 (60%) for an additional opiate (morphine, codeine, and/or oxycodone, all >50 ng/mL); and 6 (30%) for an additional benzodiazepine, either alpha-hydroxyalprazolam (>25 ng/mL) or lorazepam (>50 ng/mL).

Discussion: FXE positivity rates markedly increased in clinical urine specimens analyzed for NPS from January to April 2025 (0.128% per month) compared with 2024 (<0.001% per month). Most FXE-positive specimens were from the northeastern United States and all contained NPS from numerous NPS classes and more traditional drugs of abuse. The increased misuse of FXE is of great concern, especially when combined with other drugs that may increase the likelihood of overdose and death.

Disclosure

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

Conflict of Interest

2) Salary/Consultant

S-14

The Identification of N-Isopropyl Butylone in Postmortem Cases in Northern Virginia

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Abstract

Introduction: The emergence of synthetic cathinones on the illicit drug market over the past two decades has posed substantial challenges for forensic laboratories. Initially marketed as "legal highs," synthetic cathinones gained prominence for their stimulant-like effects. Their popularity surged around 2009, with the U.S. National Forensic Laboratory Information System reporting a drastic increase in related drug identifications. In response to the growing threat, the U.S. government enacted the Synthetic Drug Abuse Prevention Act in 2012, placing numerous synthetic substances, including synthetic cathinones mephedrone and MDPV, under Schedule I of the Controlled Substances Act. Despite these regulatory efforts, new synthetic cathinones continue to appear in forensic casework.

One such emerging compound is N-isopropyl butylone, a structural isomer of N,N-dimethylpentylone, which was the predominant synthetic cathinone identified in forensic casework in 2023 in the U.S. The Controlled Substances Section of the Virginia Department of Forensic Science (VA-DFS) first identified N-isopropyl butylone in March 2024. Since its initial detection, it has been found in 136 controlled substances cases across the state, indicating its emergence in the illicit drug supply. Between July and September 2024, the Toxicology Section of the Northern Laboratory of VA-DFS identified N-isopropyl butylone in six postmortem cases.

Objectives: This study aims to investigate the presence of N-isopropyl butylone in postmortem toxicology cases analyzed by the VA-DFS Northern Laboratory between July and September 2024. Six cases were identified to be studied with two being selected for confirmation testing due to specimen volume requirements. Specimens were evaluated for drug concentrations of co-occurring substances, case history, and manner and cause of death.

Methods: Presumptive identifications of N-isopropyl butylone were achieved through full scan gas chromatography–mass spectrometry analysis following an alkaline liquid-liquid extraction. For confirmation, a liquid-liquid extraction developed in-house targeting amphetamines was adapted by adding calibrators and controls for N-isopropyl butylone with analysis utilizing liquid chromatography-tandem mass spectrometry. The calibration range studied was 0.010-2.0 mg/L, though results are semi-quantitative as a full validation was not completed. Case studies were completed for each case with information provided by the Office of the Chief Medical Examiner.

Results: N-isopropyl butylone was presumptively identified in six postmortem cases in the Toxicology section of the Northern Laboratory of the VA-DFS during the normal course of casework. N-isopropyl butylone was confirmed present in two cases with semi-quantitative values of 0.20 and 0.021 mg/L.

Three deaths were due to drug intoxication, and three involved fatal gunshot wounds, though toxicological findings may have contributed to behavioral or physiological effects prior to death. Both quantitated cases involved death due to fatal gunshot wounds. All cases exhibited poly-drug

use with fentanyl identified in every case and stimulants present in all but one.

Discussion: The presence of N-isopropyl butylone cases underscores the known concern regarding novel psychoactive substances in forensic toxicology. As very little is known about N-isopropyl butylone, it is difficult to ascertain the exact role it is playing in these cases and the potential effects it may have with other drugs. The presence of fentanyl in all cases potentially indicates that N-isopropyl butylone may have been an adulterant circulating in the northern Virginia area between summer and fall of 2024. Since September 2024, it has not been presumptively identified in any further Toxicology cases examined, further supporting that it may have been an isolated fentanyl adulterant. Identification from the Controlled Substances section, however, contradicts this theory and indicates a more widespread prevalence, highlighting the need for further and more comprehensive testing. While the source and precise role of N-isopropyl butylone in these deaths is not fully understood, its inclusion in the toxicological profile signals its growing prominence in recreational drug use.

Disclosure

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

S-15

Comprehensive Profiling and Characterization of NPS Drugs Using Trapped Ion Mobility-High Resolution Mass Spectrometry

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Abstract

Background/ Introduction: The global proliferation of new psychoactive substances (NPS) continues to challenge forensic, clinical, and regulatory sectors due to their rapid emergence, structural diversity, and limited analytical data. Among these, isomeric compounds present a particular analytical hurdle, often evading traditional detection and identification workflows. Advanced analytical platforms capable of resolving structural and conformational complexities are essential for the comprehensive characterization of these compounds. Trapped Ion Mobility Spectrometry coupled with high-resolution mass spectrometry (TIMS-HRMS) offers a promising avenue for enhancing selectivity and confidence in NPS identification through orthogonal separation dimension and accurate mass measurement.

Objectives: This study aimed to establish a robust, high-resolution, non-targeted analytical workflow for comprehensive profiling and differentiation of 40 structurally diverse NPS compounds, including 25 isomers, using LC-TIMS-TOF-MS. By employing both data-dependent acquisition (DDA) and data-independent acquisition (DIA) strategies, we sought to evaluate the capabilities of 'TIMS-Collision Induced Dissociation-MS' and 'TIMS-Data Independent Acquisition-Parallel Accumulation Serial Fragmentation-MS/MS' modes in resolving isomeric mixtures, capturing diagnostic fragmentation patterns, and enhancing confidence in structural elucidation.

Methods: All analyses were performed on a Bruker timsTOF instrument coupled with UHPLC. The 40 NPS compounds, spanning various chemical classes such as synthetic cannabinoids, cathinones, opioids, phenethylamines, and designer benzodiazepines, were first analyzed individually to acquire baseline mobility, retention time, and fragmentation data. Subsequently, the compounds were grouped into six mixtures, carefully designed to ensure each isomer was placed in a distinct mix to prevent intra-group ambiguity. Both TIMS-CID-MS (Data Dependent Acquisition-DDA) and TIMS-DIA-PASEF-MS/MS (DIA) methods were developed under optimized conditions typically accounting for LC gradient profile, flow rate, ion mobility ramp settings, accumulation and ramp times, mass ranges, and appropriate collision energies for efficient fragmentation, to evaluate the method performance in non-targeted identification and isomeric differentiation. Samples were analyzed using a Zorbax C-18 column with a 20-min gradient elution method, alternating between aqueous phase A (5mM ammonium formate in 0.1% formic acid in water) and organic phase B (0.1% formic acid in methanol). Mobility-resolved fragment spectra were processed using Bruker DataAnalysis software, enabling 3D feature extraction (m/z, retention time, collision cross section, and intensity) and spectral deconvolution.

Results: The combined use of ion mobility separation and high-resolution MS significantly improved the detection and resolution of NPS isomers. The TIMS-CID-MS (DDA) workflow enabled selective precursor fragmentation with high spectral clarity, capturing key class-specific neutral losses and product ions useful for compound annotation. Ion mobility separation added an orthogonal layer of selectivity, effectively reducing spectral overlap and aiding in distinguishing compounds with identical m/z but different conformations.

The TIMS-DIA-PASEF approach further extended these capabilities by combining high sensitivity with broad, unbiased coverage. Leveraging the PASEF technique, ions were accumulated in parallel and fragmented serially with high speed and efficiency, enabling deeper interrogation of complex samples and improved detection of low-abundance analytes. This approach proved particularly valuable in resolving co-eluting species and confidently assigning fragments to their precursors using mobility-resolved data.

Across all experiments, sub-ppm to ~1 ppm mass accuracy was consistently achieved, strengthening the reliability of identification. Together, these workflows enabled a robust, multi-dimensional strategy for the non-targeted analysis of NPS, with enhanced specificity and resolution even in challenging isomeric contexts.

Conclusion/Discussion: This study demonstrates the potential of TIMS-HRMS platforms, particularly when coupled with DDA and DIA strategies, to overcome the limitations of conventional LC-MS/MS in the non-targeted analysis of NPS. The orthogonality of ion-mobility separation and high-resolution mass detection enhances the ability to resolve isomeric compounds and provides additional metrics for compound identification, such as Collision Cross-Section (CCS) values. These findings support the integration of LC-TIMS-TOF-MS workflows into forensic and toxicological screening pipelines, particularly for dynamic and structurally complex drug landscapes. Future work will focus on expanding the spectral library with experimentally derived CCS and MS/MS data to facilitate rapid identification in real-world case samples.

Disclosure

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

S-16

In Vitro Metabolism of *N*-piperidinyl Etonitazene for Forensic Toxicology Investigations

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Abstract

Introduction: In recent years, a wide variety of nitazene analogs have emerged in seized drugs and toxicological samples, including many with *N*-piperidinyl or *N*-pyrrolidinyl substitutions. However, their metabolic disposition is poorly understood due to their potency and illicit nature. In biological samples, metabolites serves as biomarkers for drug use and may differ in pharmacological activity, influencing overall effects. Understanding the metabolism of novel nitazenes is essential for interpreting parent/metabolite findings in forensic toxicology. Case studies and *in vitro* human liver microsome (HLM) studies have identified common metabolism pathways for nitazenes, such as *N*-dealkylation (except in ring-substituted nitazenes), *O*-dealkylation, hydroxylation, and nitro reduction. However, the specific cytochrome P450 (CYP) isoforms involved remain unclear. Since enzyme activity can be affected by genetic variability or exogeneous inhibitors, identifying the enzymes responsible for nitazene metabolism may improve both forensic interpretation and public health awareness.

Objectives: This study aimed to evaluate which CYP isoforms are responsible for the metabolism of *N*-piperidinyl etonitazene and to characterize the resulting metabolites.

Methods: Samples were analyzed using an Agilent 6530 Q/TOF-MS coupled to an Infinity Binary II LC system with an Agilent Poroshell 120 EC-C18 column (2.1 x 100 mm, 2.7 µm) and matching guard. A 12-minute gradient using 0.1% formic acid and 5 mM ammonium formate in deionized water (A) and 0.1% formic acid in acetonitrile (B) was optimized. Data were acquired in auto-MS/MS (data-dependent acquisition) mode.

Initial screening used HLMs in 500 µL reactions (n=2) with 1 mg/mL pooled HLMs, 24.5 µM analyte [BS1], and NADPH regeneration system in pH 7.4 potassium phosphate buffer. Samples were pre-incubated for five minutes, then incubated for two hours at 37°C. Reactions were quenched with cold acetonitrile containing internal standards (0.4 µg/mL isotonitazene-$^{13}C_6$ and metodesnitazene-D_4), vortexed, centrifuged, and the supernatant was diluted 1:1 with 90:10 A:B mobile phase for analysis. Negative controls (for analyte and HLMs) were run concurrently with samples.

After confirming metabolite production with HLMs, subsequent incubations were completed in 250 µL reactions using CYP3A4 or CYP2D6 bactosomes (0.05 nmol/mL enzyme, 50 µM drug, and NADPH system in buffer). Samples were quenched and analyzed after 0, 15, 30, 60, and 120-minute incubations. Control bactosomes and negative controls were assessed concurrently. To confirm CYP3A4 enzyme activity observed, inhibited samples were prepared in triplicate (n=3) with the addition of 40 µM ketoconazole alongside uninhibited (n=3) samples, which were evaluated after 120 minutes of incubation.

Results: The HLM studies were used to optimize chromatographic separation of the proposed metabolites and to develop the preferred exact mass list for auto-MS/MS acquisition for optimized detection. CYP3A4 was involved in the production of *N*-piperdinyl 4'-OH nitazene, confirmed by

accurate mass (<5 ppm), MS/MS fragmentation of the piperdinyl side chain, and comparison to a reference standard. Three other metabolites involving combinations of hydroxylation, desaturation, and *O*-desalkylation of the parent compound were suspected based on accurate mass and fragmentation, though no reference standards were available for confirmation. Inhibition studies showed metabolite relative peak areas were reduced to <50% with ketoconazole, confirming CYP3A4 involvement. Preliminary evidence also suggested CYP2D6 may contribute to the formation of *N*-piperidinyl 4'-OH nitazene and minor metabolites.

Discussion: CYP3A4 appears to play a key role in the biotransformation of *N*-piperidinyl etonitazene to its metabolites, including through the common *O*-desalkylation pathway. CYP2D6 may also be involved in this path, although further studies are pending for this isoenzyme and others. These findings help characterize potential metabolic pathways of nitazenes which is important in the context of polydrug use and interindividual variability, supporting both forensic interpretation and public health efforts related to emerging synthetic opioids.

Disclosure

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

S-17

Postmortem Benzodiazepines 6-Year Review in Franklin County, Ohio with Most Recent Contender Phenazolam

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Abstract

Introduction: Benzodiazepines have historically been prescribed as anxiolytics or hypnotics, but in recent years have become a frequent contributor to the NPS world. The pharmacodynamic properties of the benzodiazepine class are dependent on the structural makeup, leading to desirable effects for the user. At the FCFSC Office of the Coroner Division of Toxicology, benzodiazepines are screened by ELISA and LC/QTOF and confirmed by GC/MS.

Objectives: To share benzodiazepine statistics for Franklin County, Ohio for the years 2019-2024 completed by the Toxicology Laboratory on decedents admitted to the coroner's office. To highlight where the scene evidence collected played a crucial part in identifying a benzodiazepine never seen before in FCFSC casework.

Methods: A statistical review was completed for all benzodiazepine results included on the final toxicology reports between 2019-2024. Manner of death, demographics, and an itemized look of the benzodiazepine scope for the laboratory was reviewed. For the phenazolam case, analysis was completed on evidence collected from the scene and biological specimens collected from autopsy. A dilution method on the scene evidence identified drugs present qualitatively by GC/MS. Biological screening included Immunalysis enzyme-linked immunosorbent assay (ELISA) for acetaminophen, benzodiazepines, cannabinoids, cocaine, fentanyl, methamphetamine, opiates, oxycodone and salicylates partnered with a basic extract analysis on an Agilent 1260-6546 LC/Q-TOF. Reflex confirmation was performed by solid-phase extraction with derivatization and analysis by an Agilent 8890-5977C GC/MS in both full-scan and selective-ion monitoring modes.

Results: Systematic toxicological analysis (STA) was completed on 13,384 cases over 6-years to which 7,643 had a manner of death listed as accident, suicide or undetermined. Of those, 545 overdoses included a benzodiazepine on the toxicology report. Accidental overdoses accounted for 504 cases with demographics being 79.3% White, 14.8% African American, 2.3% Hispanic, and 3.3% other to which 60.7% were male and 39.2% female. The most prevalent benzodiazepines seen in the accidental overdoses were found to be alprazolam (n=137), diazepam (n=92) and bromazolam (n=74).

A case received in February 2025 resulted in the identification of a new benzodiazepine for Franklin County, Ohio. A 23 y/o white female was found unresponsive in her apartment. Phenazolam was identified by library search capabilities via GC/MS-full scan in green pill bars imprinted "S 90 3" collected from the scene. The identification was confirmed by comparison against a Cayman Chemical reference standard (item#26700). Qualitative identification of phenazolam was also found in peripheral and heart blood along with the metabolite alpha-hydroxy-phenazolam by GC/MS and LC/QTOF. The STA also identified acetone, β-hydroxybutyric acid, ketamine, norketamine, cannabinoids, and fentanyl in peripheral blood.

Discussion: A 6-year statistical review of benzodiazepines seen in postmortem casework emphasized the need for flexible analytical approaches beyond in-service methods. Identification of analytes from scene evidence is valuable knowledge that can lead the toxicology scope in a new direction. The 23-yo female's manner of death was ruled an accident with the immediate cause as a combined acute ketamine, fentanyl and phenazolam intoxication.

Disclosure

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

S-18

High Behind Bars: A Retrospective Study of Drugs Detected in Miami-Dade Correctional Facilities

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Abstract

Introduction: In Miami-Dade County, approximately 12,700 individuals are incarcerated on any given day in county, state, and federal correctional facilities. Pursuant to Florida Statute §406.11, the medical examiner is required to certify all inmate deaths that may involve use of force, drug intoxication and/or overdose, medical neglect, and/or underlying medical conditions. Therefore, an independent and thorough death investigation is essential as it provides a statutory role critical to maintaining public trust and ensuring transparency within a custodial setting. These medicolegal death investigations are multidisciplinary and incorporate law enforcement reports, medical records, social history, autopsy findings, toxicology testing, and ancillary analyses. This comprehensive approach ensures an accurate determination of the cause and manner of death. Timely and thorough toxicology testing is especially important for identifying substances that may have contributed to or caused death in a correctional facility.

Objectives: This study aims to evaluate inmate deaths over a ten-year period, with a particular focus on toxicological findings and the prevalence of novel psychoactive substances in correctional facilities.

Methods: A retrospective review was performed to identify all inmate deaths that occurred in Miami-Dade County, Florida from January 2015 through May 2025. This analysis included demographic information, cause of death, manner of death, and toxicology findings. For every case, a routine toxicological analysis was performed, including a screen for illicit substances, prescription medications, novel psychoactive substances, and over-the-counter drugs by gas-chromatography-mass spectrometry (GC-MS), liquid chromatography-tandem mass spectrometry (LC-MS/MS), and/or liquid chromatography-ion trap-mass spectrometry (LC-Ion Trap-MS^n).

Results: Between January 2015 and May 2025, there were a total of 756 inmate deaths in Miami-Dade County. Of these, 75% (n=567) were certified by the medical examiner based solely on extensive medical histories; therefore, they were not included in the study. The remaining cases underwent a comprehensive medicolegal death investigation, including a forensic autopsy and toxicology testing. Among the remaining cases, the majority of decedents included white males between the ages 40 and 69 years old; 189 inmates tested positive for drugs at their time of death. Natural deaths accounted for 54% of cases where drugs were detected, many of these deaths were due to cardiac-related conditions. Suicides (primarily hangings) and homicides accounted for the fewest number of deaths at 12% and 4%, respectively. The drugs identified in natural deaths, homicides, and suicides showed considerable variability, though they primarily consisted of prescription and over-the-counter medications. This contrasts with accidental deaths, where novel psychoactive substances were much more prevalent.

Accidental deaths accounted for 25% of inmate fatalities (48 cases), and of these, 83% (40 cases) were due to a drug overdose. Synthetic cannabinoids were the most common illicit substance found in Miami-Dade County correctional institutions. They were listed in the cause of death in half of the overdose fatalities, with 11 attributed to a single synthetic cannabinoid, primarily

5-fluoro-ADB (eight cases). Additional synthetic cannabinoids identified in prisoner fatalities were MDMB-4en-PINACA, 5-fluoro-MDMB-PICA, MMB-2201, ADB-FUBIATA, ADB-4en-PINACA, and ADB-BUTINACA. Synthetic cathinones were the second most common drug class included in the cause of death (16 cases), with N,N-dimethylpentylone most frequently identified. Additional cathinones detected included eutylone, N-ethylpentylone, alpha-pyrrolidinovalerophenone, 4-fluoro-3-methyl-alpha-PVP, N-cyclohexylbutylone, N-cyclohexymethylone, and most recently N-isopropylbutylone. All synthetic cathinone-related deaths were consistently listed alongside other illicit substances in the cause of death. Fentanyl was detected in nine cases, eight of which involved multiple substances, and fentanyl analogs (cyclopropyl fentanyl, fluorofentanyl, and para-fluoroisobutyrylfentanyl) were identified in six cases. Another prevalent drug detected in inmate deaths was cocaine which was present in eight cases, all in combination with other drugs.

Discussion: This ten-year review highlights the vital role of the medical examiner in maintaining transparency and public trust in custodial death investigations. While most deaths were natural and involved prescription or over-the-counter medications, accidental deaths were largely driven by drug overdoses, primarily from synthetic cannabinoids and cathinones. These novel substances are prevalent in correctional settings due to their potency, concealability, and ease of smuggling. Comprehensive toxicology testing remains essential for identifying emerging drug trends and preventing future fatalities in incarcerated populations.

Disclosure

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

S-19

A PCP Overdose Death in Northern Virginia: A Case Study

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Abstract

Introduction: Phencyclidine (PCP) is a Schedule II dissociative anesthetic that is no longer used medically. The use of PCP appears to be localized to certain metropolitan areas in the United States, including the Baltimore-Washington DC corridor. PCP use accounted for 4.25% of the driving under the influence of drugs cases in Virginia in 2021 and 0.57% of medical examiner cases from 2020-2024.

Objectives: The purpose of this study was to determine the actual blood concentration of PCP in a postmortem overdose as well as collecting information on events leading up to death.

Methods: PCP was extracted following the liquid/liquid alkaline extractable drugs method in the Virginia Department of Forensic Science's (VA-DFS) Toxicology Procedures Manual (https://dfs.virginia.gov/wp-content/uploads/220-D100%20Toxicology%20Procedures%20Manual-2816-683067ddaa58f.pdf). The blood sample submitted in this case was analyzed for PCP in triplicate on 1/40 dilutions using an Agilent 7890A/5975C GC-MS operated in selected ion monitoring (SIM) mode. A dilution control was prepared in the same manner and analyzed with the case sample.

Results: The average PCP concentration for the three extracted samples was 5400 ng/mL. The dilution control fell within ±20% the target concentration, which is the acceptance criteria at VA-DFS.

Discussion: Of the 141 medical examiner cases from 2020-2024 found to contain PCP, PCP was reported quantitatively in 96 blood cases with an average of 131 ng/mL. The results of this case are consistent with literature results for PCP fatalities, but higher than cases typically seen in Virginia. Dilutions of this magnitude have not previously been needed for PCP cases. In general, the number of PCP cases has decreased from 2020-2024. The subject was a 47-year-old white male who had a history of PCP use. He was seen shoveling snow and waking neighbors in January in the middle of the night. He was later found with a "foam cone" emanating from his nose. No other trauma to the body was found and all other toxicological analyses were negative. Following postmortem examination and toxicological analysis, the cause of death was determined to be PCP intoxication. Due to the elevated level of PCP in the absence of other drugs of abuse, the concentration of PCP and case history are presented here to provide behavioral information as well as autopsy findings of a PCP overdose.

Disclosure

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

S-20

2025 Update on Standards Development Activities in Forensic Toxicology

Marc LeBeau

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Abstract

Introduction: Standards significantly influence daily work in forensic toxicology. In the past six years, the Organization of Scientific Area Committees (OSAC) for Forensic Science and the Academy Standards Board (ASB) have together published 17 forensic toxicology standards. This presentation will provide an update on the current published standards, those nearing completion, and what to expect in the coming years.

Objectives: Attendees will have a clear understanding of the discipline-specific standards currently impacting forensic toxicology.

Impact: This presentation will benefit the forensic toxicology community by increasing awareness of standards development and highlighting valuable training, tools, and resources for their implementation, compliance, and broader understanding.

The Organization of Scientific Area Committees (OSAC) for Forensic Science was established to enhance the nation's use of forensic science by promoting discipline-specific standards. To achieve this, OSAC drafts standards that are then forwarded to Standards Developing Organizations (SDOs) for further development and publication. OSAC also curates the OSAC Registry of published standards, highlighting those that are high-quality, consensus-based and technically sound, creating a central repository for these vital resources.

The Academy Standards Board (ASB) of the American Academy of Forensic Sciences (AAFS) is a pivotal entity in advancing forensic science through the establishment of standards. As an ANSI-accredited Standards Developing Organization, the ASB is committed to providing accessible, high-quality, science-based consensus standards for the forensic sciences. The ASB achieves this by developing and publishing standards that adhere to the American National Standards Institute (ANSI) process. Its work directly contributes to safeguarding justice, integrity, and fairness by providing the forensic science community with essential guidelines and best practices, thereby fostering reliability and consistency across various forensic disciplines.

During this presentation, updates on standards development in forensic toxicology will be provided. Opportunities for supplemental training related to discipline-specific standards will be presented, along with additional resources and tools designed to facilitate gap analysis, compliance monitoring, and outreach efforts.

Disclosure

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

S-21

Laboratory Accreditation in Forensic Toxicology: A Different Perspective

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Abstract

Introduction: Laboratory accreditation for forensic toxicology laboratories performing postmortem, human performance and general poisoning analyses and interpretations has evolved over time. Various private entities have overseen such accreditations and recently there has been a de facto acceptance of accreditation that utilize standards that focus more on "forensic" aspects as opposed to the science of toxicology. Additionally, ISO 17025 has been applied to toxicology even though ISO 15189 is a more appropriate ISO standard. Unlike the need for auxiliary accreditation standards, e.g., AR3125 within ANAB, the majority of laboratory-related criteria are spelled out in ISO 15189. For example, the table of contents of this latter accreditation includes sections on laboratory governance, personnel, facilities and environmental conditions, laboratory equipment, calibration and traceability, reagents and consumables, service agreements, pre-examination processes, examination processes, post-examination processes, non-conforming work, complaints, and management requirements. Human performance toxicology, postmortem toxicology and general poisoning all represent a form of diagnosis of the poisoned patient. In these respects, toxicological analyses support the diagnosis of the patient. By extension, this makes results of analyses of samples from such patients clinically focused. Therefore, accreditation of toxicology laboratories may more appropriately be performed by clinical accrediting bodies. This presentation will lay out the argument for such accreditation and the utilization of ISO 15189 for toxicology laboratories.

Objectives: The audience will understand that toxicological analyses are used to support diagnoses of patients and subsequently that clinical laboratory accreditations, including ISO 15189 together with ASB standards, are appropriate for forensic toxicology laboratories.

Methods: Information was gathered through both open and closed (i.e., paid subscription to ISO 15189 standards) sources to assess differences between traditional forensic toxicology accreditations and other options. Further, one laboratory's experiences with both accreditations were assessed as well as information gathered from 3 forensic toxicology laboratories that have already transitioned to clinical accreditation.

Results: Both theoretical and active accreditation of forensic toxicology laboratories utilizing clinical standards is practical and perhaps more relevant than current accreditation processes. Through discussion with forensic toxicology laboratory directors that are using clinical assessment currently, there was a general consensus that no decrement in quality has been noticed with some stating that they believe quality has improved. This makes sense given the relevance of clinical standards to toxicological analysis in diagnosing patients. From the author's experience with both ISO 17025 and ISO 15189, it is my experience that the latter has significantly greater relevance to the practice of forensic toxicology in both substance and instruction.

Discussion: Forensic toxicology laboratories perform analyses in support of clinical diagnoses whether that be postmortem, human performance or general poisoning concerns. The current process for accreditation of forensic toxicology laboratories pushes such laboratories into standards that do not take into account the unique aspects of the science. ISO 17025 is a

materials-based standard with no particular suitability for toxicology laboratories, whereas ISO 15189 is focused on clinical laboratories that include toxicology. ISO 15189 would not be appropriate for crime laboratories that perform materials analysis, e.g., drug chemistry, arson evidence, etc. Several laboratories have already made the transition to clinical standards for postmortem toxicology and have reported no decrement in quality. To accommodate some of the concerns regarding arguable specific "forensic" needs, implementation of ASB standards adequately fills such perceived void. As a result, acceptance of different, yet suitable, accreditation processes should be welcomed by the forensic toxicology community.

Disclosure

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

Conflict of Interest

I am a salaried employee of NMS Labs.

S-22

Training That Works: Feedback-Driven Refinement of a Comprehensive QTOF Training Program

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Abstract

Introduction: In recent years, laboratories have begun transitioning from traditional screening techniques such as immunoassay and full scan mass spectral acquisition to high resolution mass spectral techniques, such as liquid chromatography-quadrupole-time of flight mass spectrometry (LC-QTOF/MS). However, to maximize the full potential of high resolution mass spectrometry (HRMS) for drug identification, laboratory staff require extensive training in instrumental operation and interpretation of complex data. As with the introduction of any new technology, levels of experience and knowledge among staff differ. Development of a sufficient training program for all staff is critical for fundamental understanding of theory, confidence in operation and maintenance, proficient review of analytical data, and competency for authorization.

Objectives: To present the QTOF training program developed at the Harris County Institute of Forensic Sciences to include (1) the training process, (2) evolution of the program, and (3) employee feedback.

Methods: Staff were divided into four small groups based on position title and experience level. Each group underwent a three month training program that included (1) reading assigned materials, (2) receiving lectures and practical demonstrations of QTOF theory, instrumentation operation and maintenance, method parameters, and data review and reporting, (3) performing instrument maintenance under a trainer's supervision, (4) analyzing data with and without supervision, (5) performing technical review, (6) completing a written exam and (7) an oral exam. The data analysis component included evaluating 100 authentic casework samples to practice software familiarization, acceptance criteria evaluation, and making reporting or reflex testing decisions. Trainers reviewed all work for satisfactory completion. Post-training, trainees completed two surveys to gather (1) feedback for future groups and (2) detailed information on program materials and application to casework.

Results: Twenty-one staff members responded to one or both surveys. Prior to training, 62% of staff members had <1 year of experience with HRMS. As a result of feedback from the first group of trainees (senior toxicologists, n=9), group size, time allotted for tasks and frequency of review (to allow for consistent trainer feedback) were adjusted for subsequent groups. Content was adjusted to optimize group learning, facilitate understanding of practical information, and highlight critical information to complete training tasks and casework. Of the training activities provided, most respondents (60%) identified hands-on activities as the most beneficial, followed by lectures (25%) and observation (15%). Trainees found the least benefit from the written exam. Comfort level with QTOF theory, maintenance and operation, and data review were above average for all staff post-training. Of 12 staff members who completed a casework batch post-training, 75% felt sufficiently prepared by the training program.

Discussion: The QTOF training program was designed to incorporate different learning styles (visual, observational, and hands on). Readings and recorded lectures/processes were useful references for staff during unsupervised tasks. Sufficient practice was beneficial for trainees as

many indicated repeated exposure to boost confidence performing data review. Feedback was critical for both trainees in the learning process and trainers in the evolution of the program. Developing a comprehensive training program for new technologies, such as LC-QTOF/MS, is critical for forensic toxicology laboratory effectiveness and workflow.

Disclosure

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

S-25

The Pharmacology of Kratom, Patterns of Use, and Growing Diversity of Kratom Products in the US: Implications for Forensic Toxicology Investigations

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Abstract

Introduction: The novel psychoactive substance kratom is increasingly reported in both impaired and postmortem cases. The number of reported cases and diversity of clinical presentation appears to indicate an increasing consumption of kratom by the general US adult population with an estimated lifetime prevalence ranging from 0.6 to 9.0%.

Objectives: This presentation provides an update on the complex pharmacology of kratom alkaloids to inform toxicologists about the diverse symptom presentation. The use patterns of kratom products is discussed to provide a distinction when evaluating kratom exposures for contribution or causality to impairment or fatal outcomes. The increasing diversity of kratom products is briefly discussed and its potential link to expected bodily fluid concentrations.

Methods: The current literature is reviewed to adequately contextualize and summarize our understanding of kratom both in the clinical and forensic toxicology setting.

Results: Kratom alkaloids present with a diverse range of pharmacological actions, including opioid, adrenergic, serotonergic, and adenosine receptor effects. Though the primary alkaloid mitragynine is used to indicate or correlate kratom exposure, other alkaloids and metabolites may contribute substantially to the toxicity of kratom products. Kratom is primarily used for self-treatment of various health conditions including pain, mental conditions, and as a harm reduction tool to reduce withdrawal effects from other substances of abuse, in particular opioids, alcohol, and stimulants. Adverse effects, including impairment, appear to be correlated with novel kratom products, especially extracts and concentrates that may contain up to 40 times the alkaloid amount of a native leaf product. Furthermore, single kratom alkaloid products such as 7-hydroxymitragynine and mitragynine pseudoindoxyl are being marketed that may be associated with substantially increased risk of adverse effects and fatality.

Discussion: As part of the forensic investigation, kratom should be considered as either a contributory or causative substance. In order to facilitate a comprehensive evaluation of the individual case, investigators and forensic toxicologists should request samples of the kratom products to determine whether kratom exposure contributed to impairment or a fatality.

Disclosure

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

Conflict of Interest

I am a paid expert consultant on legal cases involving kratom exposure. I have also received honoraria for presentations on kratom and related substances.

S-26

Safety and Tolerability of Mitragyna Speciosa MitraPlus™ (Kratom Extract) in Human Volunteers in the KAPTURE Study Following Controlled Administration of Single and 15 Multiple Daily Extract Doses

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Abstract

Introduction: There are no available safety data on controlled administration of kratom extract, a more concentrated full kratom preparation with increasing US prevalence.

Objectives: To determine the safety and tolerability of MitraPlus kratom extract in human volunteers in a randomized, placebo-controlled, double blind study.

Methods: Over 31 clinic visits, 12 volunteers ingested single 1, 3 or 6 capsule doses containing active 9.87, 29.6, or 59.2 mg mitragynine extract or matched placebo. Full safety monitoring occurred for 10 days. Next, the same volunteers received the same dose for 15 consecutive days with 23 day safety monitoring. Treatment emergent adverse events (TEAE) monitoring, metabolic and hematology tests including for hepatic and kidney function, respiratory rate, SpO_2, ECG and vital signs occurred throughout and mitragynine withdrawal was assessed after 15 multiple doses.

Results: There were no serious adverse events or deaths. The percentages of participants taking Mitra-Plus reporting TEAE increased with dose. There were 8 early terminations due to AE, 1 after 9.87 mg (due to a moderate TEAE of sinus tachycardia, but the ECG was asymptomatic) and 7 after 59.2 mg mitragynine extract (due to nausea and vomiting, 2 withdrew voluntarily due to TEAE not deemed clinically significant by the PI, and 4 due to doubling of their baseline ALT and AST). All early termination TEAE resolved after product cessation. For abuse potential-related TEAE after the single dose, somnolence was most commonly reported, increasing from 1 to 5 participants from mid to high dose. There were no reports of euphoric mood, the most relevant abuse potential TEAE. After multiple doses, somnolence also was most commonly reported, increasing from 3 to 6 participants from mid to high dose. Euphoric mood was reported in 2 and 3 participants in mid and high dose groups. Three participants reported mild withdrawal-related TEAE of lacrimation increased, anxiety, abdominal pain, drug withdrawal syndrome or restlessness; only one after the highest multiple dose. Any shift from normal to abnormal high or low laboratory results were recorded for 13 variables over 31 visits, with no consistent trends in most clinical chemistry and hematology parameters. Five participants at the highest dose shifted from normal to high and 4 participants shifted from normal to low results across all chemistry variables across all visits; ALT and AST had the most chemistry shifts. For hematology shifts at the highest dose, 3 shifted to abnormal high and 4 to abnormal low results. There were no reports of euphoric mood following any single MitraPlus dose; after multiple 59.2 mg extract doses, 3 participants reported mild euphoric mood on several days. The predominant abuse potential-related TEAE were mild somnolence and dizziness. There were no withdrawal related TEAE after the highest extract dose. There were no liver enzyme increases >2x baseline after single doses, and after multiple doses, 1 receiving 29.6 mg mitragynine and 4 receiving 59.3 mg increased. There were no clinically significant effects on kidney function. There were no TEAE that were reported in more than 1

participant in the follow-up phase.

Discussion: MitraPlus extract was generally well tolerated across the range of 9.87-59.2 mg mitragynine in kratom extract, with almost all TEAE mild in severity, although 4 of 13 participants were withdrawn in the highest dose group due to liver enzymes >2x upper limit of normal; none met the FDA's definition of drug-induced liver injury (DILI) that encompasses clinical outcomes of asymptomatic liver enzyme increases to acute liver failure. These are the first safety and tolerability data for MitraPlus or any kratom extract.

Disclosure

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

Conflict of Interest

I am a consultant to NP Pharma that sponsored the clinical study. I helped design the study, analyzed the data and am presenting and publishing the results. Drs. Bothmer, Hudzik, and Henningfield are all pharmacologists who assisted analyzing the study and publishing results. Dr. Swift is an employee of NP Pharma. The study was approved by Health Canada and the Advarra IRB.

S-27

From Leaf to Lab: The Challenges of Kratom-Related Deaths

Justin Brower, Donna Papsun, William Schroeder

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Abstract

Introduction: Kratom is an herbal substance derived from the leaves of the tropical evergreen tree *Mitragyna speciosa*. It has a long history of use in Southeast Asia, where users prepare decoctions or teas for consumption. Over the last decade, kratom has gained popularity in the United States. At low doses, kratom has stimulant-like effects, while at higher doses, it shows CNS-depressant effects. Its opioid-like properties drive its use, with many individuals taking it to relieve chronic pain or to ease opioid withdrawal symptoms. Additionally, kratom's popularity is bolstered by its legality; as an herbal supplement, it is minimally regulated by the FDA and is not classified as a controlled substance by the DEA, making it legal at the federal level.

While there are many documented benefits of kratom, it is not without risks or potential for abuse, particularly with the advent of non-traditional preparations, such as concentrated "shots" and powders. Among the numerous compounds found in kratom, the primary alkaloid is mitragynine, which functions as a partial agonist on opioid receptors. Although the pharmacology of mitragynine and other kratom alkaloids is intricate and not fully understood, it has been implicated in many drug overdose deaths, either alone or in combination with other substances.

Objectives: Interpreting mitragynine in postmortem cases is challenging, owing to its pre-analytical and analytical issues, as well as the physical properties of the drug itself. The aim of this talk is to discuss these challenges through case studies and provide recommendations to aid in their interpretation.

Methods: Analytical data collected over a 7-year period from 2018 through 2024 from postmortem casework were analyzed for cases where confirmation testing for mitragynine was performed. Routine drug screening for mitragynine was achieved using High-Performance Liquid Chromatography/Time of Flight-Mass Spectrometry (LC/TOF-MS) with a single-point calibrator and cutoff at 10 ng/mL. Confirmation testing for mitragynine was performed by High-Performance Liquid Chromatography/Tandem Mass Spectrometry (LC-MS/MS) with a reporting limit of 5 ng/mL.

Results: Over the 7-year period, the number of mitragynine cases has increased from 849 to 1,489 cases, rising nearly every year. Along with the increased prevalence, mitragynine's mean and median concentrations have increased from 350 and 120 ng/mL in 2018, to 653 and 220 ng/mL in 2024, respectively. But perhaps one of the most startling and toxicologically significant trends, however, is the increase in cases greater than 1000 ng/mL, a concentration which could be lethal on its own or in combination with other drugs. In 2018, there were 70 cases with concentrations greater than 1000 ng/mL, representing 8.9% of mitragynine-positive cases, which increased dramatically to 285 cases and 19.1% in 2024.

Discussion: Though there are very few deaths attributed to kratom where it has traditional ethnobotanical use, the same cannot be said in the United States, where the same dried leaf material is readily available, as well as capsules, tablets, extracts, concentrates, gummies, and bottled beverages. The increase in kratom products, coupled with an increase in its popularity, has resulted in a rise in mitragynine in postmortem casework, mean concentration, and the

number of cases exceeding 1000 ng/mL. With these increases, it is even more imperative to adequately consider the role of kratom and mitragynine in postmortem cases so that we do not under- or over-attribute its significance in the cause of death.

Keywords

Mitragynine, kratom, postmortem

Disclosure

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

S-28

7-Hydroxy Mitragynine and Related Kratom Alkaloids in Forensic Casework

Alex Krotulski[1], Max Denn[1], Justin Brower[2], Donna Papsun[2], Barry Logan[1,2]

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Abstract

Introduction: Mitragynine is a plant alkaloid and the primary psychoactive component of Kratom (Mitragyna speciosa). 7-Hydroxy mitragynine is a structurally similar alkaloid found naturally in Kratom but in smaller amounts and is also a metabolite of mitragynine. In 2016, the U.S. Drug Enforcement Administration (DEA) issued a notice of intent to schedule mitragynine and 7-hydroxy mitragynine; however, the DEA ultimately withdrew their proposed rule, leaving these Kratom alkaloids unscheduled and uncontrolled in the U.S. In recent years, concurrent with the rise of "smoke shops", commercial sale of Kratom products, high dose mitragynine preparations, and now marketed 7-hydroxy mitragynine products have surged. These marketed products include chewable tablets, concentrates, extracts, edible materials (e.g., gummies, ice cream cones), and beverages, with highly elevated 7-hydroxy mitragynine contents compared to Kratom products.

Objectives: This presentation aims to provide insights into the newly diverse mitragynine alkaloid market, showcasing test results from drug product analysis and medicolegal death investigations involving 7-hydroxy mitragynine labeled products.

Methods: Drug products (e.g., powder, pills) were collected from partnering organizations or purchased from local smoke shops. Comprehensive drug analysis was performed by gas chromatography mass spectrometry (GC-MS) and liquid chromatography quadrupole time-of-flight mass spectrometry (LC-QTOF-MS). Biological specimens from medicolegal death investigations were submitted by partnering forensic toxicology laboratories and medical examiner or coroner offices. Comprehensive toxicological analysis was performed by LC-QTOF-MS. Data processing included targeted, concurrent spectral identification of mitragynine, 7-hydroxy mitragynine, and mitragynine pseudoindoxyl. Suspect screening was performed for mitragynine isomers (e.g., speciogynine, speciociliatine, mitraciliatine) and additional Kratom alkaloids (e.g., paynantheine, ajmalicine, mitraphylline, corynantheidine). 7-Hydroxy mitragynine and mitragynine pseudoindoxyl were indistinguishable by GC-MS; therefore, analysis via LC-QTOF-MS was required for identification and differentiation.

Results: Six drug products marketed as containing 7-hydroxy mitragynine were tested. Five products contained 7-hydroxy mitragynine as the primary component. One product contained mitragynine pseudoindoxyl as the primary component with 7-hydroxy mitragynine as the secondary component. All products contained at least detectable amounts of mitragynine and mitragynine pseudoindoxyl, as well as some other Kratom alkaloids; however, the ratios of the substances varied.

7-Hydroxy mitragynine was added to our toxicology testing scope in 2016. In early 2025, our laboratory began receiving inquiries from forensic collaborators about cases suspected of being 7-hydroxy mitragynine intoxications. To date, three cases with history of 7-hydroxy mitragynine product ingestion were received and analyzed qualitatively (with quantitation pending) – additional suspected cases are pending receipt. All cases tested positive for 7-hydroxy mitragynine, in

addition to varying levels of mitragynine and mitragynine pseudoindoxyl (similar to the drug products results). In one case, a decedent was found in bed alongside a product labeled “Ohms”. The decadent had no history of illicit drug use. Autopsy showed significant pulmonary congestion, some foamy fluid in the airway, and no other major abnormalities. Initial toxicology testing reported mitragynine (<5 ng/mL) but 7-hydroxy mitragynine was not included in the scope. The presence of 7-hydroxy mitragynine was confirmed during secondary analysis. The blood specimen also contained sertraline, lamotrigine, gabapentin, hydroxyzine, and lorazepam. The manner of death was accident, and the cause of death was probable 7-hydroxy mitragynine toxicity (prior to secondary toxicology testing).

Discussion: Mitragynine is metabolized to 7-hydroxy mitragynine and further to mitragynine pseudoindoxyl; however, the *in vivo* presence of these alkaloids is often unclear as both can arise from Kratom itself. 7-Hydroxy mitragynine and mitragynine pseudoindoxyl are reportedly 10x and 100x more potent than mitragynine, respectively. Testing for these two alkaloids is extremely limited based on published reports, and mitragynine and 7-hydroxy mitragynine exhibit poor stability, especially in biological matrix. Forensic cases involving these Kratom alkaloids are increasing yet underreported.

Disclosure

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

Conflict of Interest

Grant/Research

S-29

Kratom Prevalence in Dallas County from 2018 to 2024

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Abstract

Introduction: Kratom refers to the *Mitragyna speciosa* tree and to the products derived from isolation of its chemical compounds. The use of the predominant psychoactive alkaloid, mitragynine, has increased over the years in the United States due to its stimulant and opioid-like effects. A second alkaloid of interest is 7-hydroxymitragynine, which is also the primary metabolite of mitragynine. Currently, Kratom is legal to purchase and sell in Texas for those 18 and older but is subject to specific regulations since 2023 under S.B. 497. The requirements for Kratom processors and retailers include limits on levels of 7-hydroxymitragynine and ensuring the product is not contaminated or adulterated with non-Kratom substances.

At the Dallas County Southwestern Institute of Forensic Sciences (SWIFS), mitragynine was reported qualitatively from a comprehensive drug screen by liquid chromatography time of flight mass spectrometry (LC-QTOF-MS) from 2018 to late 2022. In August 2022, mitragynine was added to an existing quantitation confirmation method by LC-MS/MS with a range of 10.0 – 1,000.0 ng/mL. 7-hydroxymitragynine was added to the LC-QTOF-MS drug screen in May 2022. Due to the known instability of 7-hydroxymitragynine it was not added to the LC-MS/MS confirmation method and continues to be reported qualitatively from the LC-QTOF-MS drug screen.

Objectives: To assess the prevalence of Kratom use in Dallas County and surrounding areas from 2018 to 2024 by evaluating toxicological and demographic information related to mitragynine and 7-hydroxymitragynine results in postmortem and antemortem casework. Case studies will be presented.

Methods: Case samples submitted to the Toxicology Laboratory at SWIFS between January 1, 2018, and December 31, 2024, were reviewed for positive mitragynine and/or 7-hydroxymitragyinine results in blood and urine. The demographics of the individuals (race, sex, and age) were evaluated for medical examiner casework, as well as cause and manner of death. Age was evaluated for driving while intoxicated (DWI) casework; sex and race data were not available.

Results: Over the 7-year period, there were 271 cases that were positive for mitragynine and 28 cases positive for 7-hydroxymitragynine; one case was positive for 7-hydroxymitragynine only. In 2018 there were 16 positive cases for mitragynine/7-hydroxymitragynine and in 2024 the number had increased to 62 positive cases. There were 243 postmortem cases, 25 DWI cases, and 3 sexual assault cases. The average mitragynine concentration in blood for all cases was 440.1 ng/mL (range 11.2 – 5,334.5 ng/mL, n=109). Results in urine were reported qualitatively.

For postmortem casework, users were 80% male and 20% female. Race demographics showed users were 86% White, 9% Hispanic, 4% Black, and 1% Asian. The average age was 39 years and ranged from 20 days to 73 years. For DWI casework, the average age was 37 years and ranged from 25 to 61 years.

Out of the 243 postmortem cases, the medical examiner listed mitragynine in the cause of death in 109 cases, with 105 cases determined as “Accident” and 4 as “Suicide”. The cause of death was

attributed to mitragynine alone in 7 cases. All other cases were determined to be toxic effects of mitragynine and at least one other drug, most commonly fentanyl.

Discussion: Kratom positive cases at SWIFS, indicated by the finding of mitragynine and/or 7-hydroxymitragyinine, have steadily increased over the past 5 years. Evaluation of cases show the primary user of Kratom are white males in their late 30s. Unsurprisingly, Kratom is typically used with other drugs of abuse and is frequently associated with the cause of death for postmortem cases.

There is currently a proposed state bill that seeks to add Kratom to Penalty Group 1 of the Texas Controlled Substances Act. If passed, it would make the production, manufacturing, and selling of Kratom a state felony. While the addition of Kratom to the Texas Controlled Substances Act would primarily impact controlled substances laboratories, it is important for toxicology laboratories to monitor the prevalence of Kratom for continued evaluation for public health and safety concerns.

Disclosure

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

S-30

The Role of Mitragynine Versus Other Drugs in Kratom Related Deaths

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Abstract

Introduction: Mitragynine is the major active component of the plant *Mitragyna speciosa*, commonly known as kratom. In low doses, it has a stimulant effect, whereas there are sedative and opioid-like effects after high doses. The use of kratom is widespread in Sweden, and for a short while, it appeared as an herbal mixture laced with another mu-opioid receptor agonist, O-desmethyltramadol. This formulation, named Krypton, was implicated in a series of fatal acute overdoses, which prompted Sweden to schedule mitragynine as a narcotic drug in 2011. However, the plant material was not scheduled, and over the years the sales of kratom escalated to a point where the Swedish Health authorities became concerned. Reports from the National Poison Center as well as kratom related deaths reported from the National Board of Forensic Medicine led to the scheduling of the plant *Mitragyna speciosa* in March 2025. Advocates for the medicinal or recreational use of kratom plant material claim that kratom itself is safe and other drugs are the main causes of death in polydrug fatalities in which mitragynine was identified. To confirm or refute this claim, all deaths where mitragynine was detected were investigated.

Objectives: To investigate the role of other drugs in deaths involving mitragynine and to compare blood concentrations in mono-, mixed-, and non-intoxications.

Methods: All forensic autopsy cases between 2018 and 2024 where mitragynine was detected were included. Demographics, cause of death (CoD), manner of death (MoD), and toxicological findings were retrieved from the National Board of Forensic Medicine database.

Results: In total, 67 cases were identified. Males constituted 92% of the study population. The mean age was 34 years. The MoD was accident in 54% of the cases, suicide in 19% and the remaining were undetermined. The most common CoD was intoxication with 51 cases of which 14 were monointoxications with one with heroin and 13 with mitragynine as CoD. Mitragynine was also considered contributing to the CoD in 26 of the 37 poly drug fatalities.

The most common combination of drugs together with mitragynine were benzodiazepines present in 70% of the mixed intoxications with alprazolam and diazepam dominating. In 62% of mixed intoxications, other opioids were present with morphine and buprenorphine dominating. A fentanyl analog, cyclopropylfentanyl, was only found in two cases in accord with the overall low prevalence of fentanyl and fentanyl analogs in Sweden.

Femoral blood concentrations (in µg/g) ranged from 0.14-4.9 (mean 2.2) in mitragynine monointoxications, from 0.03 to 5.7 (mean 0.85) in mixed-intoxications and from 0.01 to 1.9 (mean 0.45) in cases where the CoD was not classified as intoxication. Significant differences were observed between mean concentrations for mono- vs. mixed-intoxications ($p=0.002$) and mono-intoxication vs. non-intoxications ($p=0.0005$).

Discussion: From this case series, it is clear that mitragynine can cause fatal intoxications. However, in most cases it was not obvious whether these were from intake of mitragynine as a substance or as kratom. Central nervous system depressants, mainly benzodiazepines, followed by opioids, were the most prevalent co-findings with mitragynine. This complicates assigning

the major contributor to the CoD but does suggest that such combinations may increase the risk of severe intoxication even at lower mitragynine concentrations. It may be worth noting that all monointoxications were accidents. In conclusion, the study results show that mitragynine often is combined with other central nervous system depressants that may have additive effects but that, when present at high concentration in blood, mitragynine may be the single CoD.

Disclosure

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

S-31

The Kratom Footprint: 2024 Postmortem Mitragynine Trends in Travis County, TX

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Abstract

Introduction: Mitragynine is the active alkaloid found in a species of a tropical plant native to Southeast Asia, known as *Mitragyna speciosa*, most commonly known as "Kratom." At lower doses, mitragynine gives stimulant-like effects but higher doses are associated with opioid-like effects due to activity at the μ-opioid receptor. Though mitragynine has ceremonial and traditional footprints, mitragynine misuse in Western countries is on the rise due to lack of regulation and its psychoactive properties which include euphoria, relaxation, sedation, and analgesia. As a result, mitragynine cases are steadily increasing in medicolegal death investigations despite debates on its toxicity. Limited quantitative reports of mitragynine in forensic casework exist due to its instability and the presence of multiple diastereomers that, if not chromatographically resolved, may provide inaccurate concentrations. To better understand the pharmacology and toxicology of mitragynine, it is important for laboratories to quantitate mitragynine in routine postmortem casework to investigate its contribution and role in drug-related deaths.

Objectives: Given the rise of mitragynine in the United States and lack of published studies, especially those including large data sets, we aimed to investigate the prevalence and characteristics of mitragynine in Travis County, TX and surrounding areas over the last year. Additionally, the toxicological findings from mitragynine-only postmortem cases, and other interesting mitragynine-related drug toxicities will be highlighted.

Methods: Postmortem cases submitted to the Travis County Medical Examiner (TCME) for toxicology testing between January 1, 2024 and December 31, 2024, were queried to identify the total number mitragynine-positive deaths reported during this timeframe. Demographic (sex, race, age) and toxicological (mitragynine concentrations and polysubstance trends) information was characterized for all mitragynine-positive cases, including mitragynine-related drug toxicities and non-contributory mitragynine cases.

Results: Mitragynine was identified in 46 deaths in 2024, accounting for 1.9% of postmortem cases submitted for toxicology testing (n=2427). Mitragynine-positive cases primarily occurred in white males (85%) in their early-40's. Concentrations ranged from 10-8600 ng/mL, with mean±SD (median) concentrations of 838±262 (805) ng/mL; concentrations <LOQ (10 ng/mL) were set to 10 ng/mL for statistical analyses. Of the 46 mitragynine-positive cases, 52% were ruled accidental (n=24), of which, 75% (n=18) were considered mitragynine-related drug toxicities. In only two postmortem cases was mitragynine attributed as the sole cause of death; postmortem blood concentrations were 3500 and 6000 ng/mL. Mean mitragynine concentrations from drug-related toxicities (1720 ng/mL) were significantly higher (p<0.05) than those found as incidental findings in non-contributory mitragynine-positive cases (228 ng/mL), with median concentrations of 530 and 41 ng/mL, respectively. Polydrug use was present in 16 of 18 mitragynine-related drug toxicities, with opioids (67%), specifically fentanyl (61%), and benzodiazepines (39%) representing the most concurrently identified drugs and/or drug classes. Examining all mitragynine-positive cases, the most commonly identified drug classes involved opioids (37%), benzodiazepines (30%), and amphetamines (26%).

Discussion/Conclusion: In the last year, mitragynine-positive postmortem cases were observed in almost 2% of toxicology cases submitted to the TCME, emphasizing its potential public safety threat among this population. With limited published toxicological studies involving mitragynine, and the high propensity for polysubstance use, it is difficult to understand the true impact mitragynine plays in medicolegal death investigations. Although each case should be evaluated on its own merits, based on this study, mitragynine concentrations between 500-1000 ng/mL may need to be scrutinized as contributory to death, whereas concentrations >1000 ng/mL may be more frequently associated with mitragynine-related fatalities. Additionally, with its increased presence recreationally, the variability in contents among commercial kratom products, and the enhanced risk for drug toxicity with polysubstance use (especially other CNS depressants), there is a continued need for laboratories to adapt their testing methodologies to include mitragynine to better understand its toxicological impacts.

Keywords (3)

mitragynine, postmortem toxicology, NPS

Disclosure

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

S-32

Evaluating Abnormally High Methamphetamine Concentrations and Metabolite Ratios in Driving Under the Influence of Drugs Cases

Allen Mello, Lindsey Vosters

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Abstract

Introduction: The Wisconsin State Laboratory of Hygiene (WSLH) processes over 20,000 biological samples per year for the presence of alcohol and drugs involving Operating While Intoxicated (OWI) cases as well as postmortem investigations. Of these samples, methamphetamine (MAMP) is consistently in the top five most encountered drugs in samples that are tested. Methamphetamine undergoes N-demethylation to produce amphetamine (AMP) at approximately 7% of the administered MAMP dose. Out of the samples reported positive and tested for MAMP in a short time frame of November 2023 to October 2024, four specific samples were noted as being extraordinarily high.

Objectives: Due to the extremely elevated concentrations tested in a short period, it was our goal to understand why these samples were outliers in comparison to the majority of samples tested by WSLH. Reports of each case and quantitative MAMP and AMP values were evaluated to determine if there were any commonalities between the specimens in terms of route of administration. It was also of interest to assess the concentration of AMP in relation to MAMP to both reference the metabolite ratios in the present literature and compare those ratios in the literature to the ratios seen in the elevated samples.

Methods: Methamphetamine and amphetamine are extracted using a liquid-liquid extraction (LLE) with a 4:1 ratio of butyl chloride:chloroform and trifluoroacetic anhydride (TFAA) derivatization agent. Separation, detection, and quantitation utilizes a Gas Chromatograph with a Mass Selective Detector (GC-MSD). The calibration curve used to quantitate MAMP and AMP has a limit of detection of 20 ng/ml and an upper limit of 1,000 ng/ml. Steps were taken to corroborate the previous findings in the literature by evaluating the ratio of AMP:MAMP in a review of samples tested by WSLH from January 2020 to December 2023. It was then possible to compare the AMP:MAMP ratios in the high concentration samples to both literature findings and other samples with MAMP and AMP present, previously tested by the lab.

Results: Four cases of interest were highlighted due to the high concentration of MAMP. Each case was at least nine times the highest calibrator with concentrations of 9,500 ng/mL, 12,000 ng/mL, 16,000 ng/mL, and 16,000 ng/mL. Reports were gathered from law enforcement to determine if there was a correlation between these cases. Based on law enforcement reports, it was determined that there were similarities between the route of administration and purpose of administration, in addition to the high concentrations seen. The individuals in each case had orally ingested their MAMP to conceal the evidence of illicit material during a traffic stop, ultimately yielding severe physiological responses. The presence of these high concentrations inspired a review of MAMP and AMP quantitative values seen at the WSLH from January 2020 to December 2023. AMP concentrations in the elevated case samples were approximately 1-3% of the MAMP concentrations. These AMP concentrations were lower than the supported 7% of the MAMP referenced in the literature. The evaluation of casework samples from January 2020 to December 2023, within normal calibration range, supports the literature, falling within 7-13% AMP:MAMP metabolism ratio.

Discussion: Upon review of the cases, each subject had been stopped for a traffic violation with signs of impairment already present, orally consumed methamphetamine to dispose of evidence, and throughout the stop/investigation, physically deteriorated with increasing CNS stimulant symptoms.

The four cases reviewed illustrate highly elevated MAMP concentrations from oral ingestion. The 1-3% AMP:MAMP ratio is lower than the accepted 7% which may indicate recent administration of MAMP. After ingestion in all cases, the subjects produced prominent CNS stimulant effects such as elevated heart rate, profuse sweating, rigid body movements, and difficulties standing and speaking. Methamphetamine use continues to pose a risk to the safety of drivers occupying Wisconsin's major roadways.

Disclosure

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

S-33

N-2-O(h) No! The Rise of Nitrous Oxide in DUID Casework and Best Practice Recommendations

Kari Midthun, Amanda D'Orazio, Jolene Bierly

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Abstract

Background/Introduction: The rise of nitrous oxide (N_2O) misuse in recent years is no laughing matter. From its widespread use in industries, including medical/dental, food and automotive, N_2O cartridges and tanks are readily available in a variety of sizes and even flavors. Inhalant recreational use/misuse is well known; however, information is sparse when it comes to N_2O and impaired driving investigations. As an anesthetic, N_2O gas can produce feelings of euphoria, mood changes, relaxation, disorientation, confusion, and loss of coordination – effects which are problematic to safe driving. The nature of N_2O makes it incredibly challenging to analyze in biological specimens. Rapid elimination of N_2O via exhalation requires that collection be performed quickly following a driving under the influence of drugs (DUID) incident. Additional N_2O loss may occur during specimen handling, testing, and storage.

Objectives: This study was performed to evaluate the prevalence of N_2O in recent DUID submissions from 2022 through early 2025 following redevelopment of the test method. Based on case histories and discussions with investigators, both law enforcement (LE) and postmortem (PM), best practice recommendations and considerations will be provided to help address pre-analytical factors and improve N_2O detection capabilities throughout the investigative process.

Methods: Quantitative analysis in blood was performed using headspace-gas chromatography-mass spectrometry (HS-GC-MS). Briefly, samples, fresh calibrators, and controls were prepared in headspace vials spiked with an internal standard of carbon monoxide (CO). A CO liberating reagent (1.5% saponin in 1 M sulfuric acid) was also added to increase N_2O recovery and detection. Vials were shaken for 40 min at room temperature before analysis. Results were reported from a calibration range of 1.8-180 mcg/mL with a reporting limit of 1.8 mcg/mL.

Results: From January 2022 through February 2025, the laboratory analyzed more than 1200 blood samples for N_2O. Of these, 48 cases were clearly identified as DUID-related by their submission paperwork, with 23 cases (47%) reporting positive N_2O results (median 8.9 mcg/mL, range 2.0-51 mcg/mL). Demographics show a median age of 29 years (range 16-55 years).

Follow up communications with LE provided memorable case histories, many involving vehicular crashes, and familiar scene findings. Common observations consistent with N_2O impairment included disorientation, confusion, drowsiness, unsteadiness, nodding off, slurring words, and slowed body movements. If performed, standard field sobriety tests (SFSTs) were typically completed with minimal infractions unless polydrug findings were present. The decision to test for N_2O was often based on eye-witness observation of use and/or the presence of N_2O containers. Minimizing the time from incident to collection (i.e., 90 min or less) was also significant to detecting N_2O.

Discussion: While inhalant use is present in the driving population, the very nature of N_2O can make collection and detection difficult. Additional communication with submitting agencies highlighted a lack in standardized approach for collection and handling of suspected inhalant

casework, prompting education on best practices. Recommended best practices to address pre-analytical factors include minimizing the time between incident and sample collection, selecting proper collection containers that minimize headspace, and ensuring containers are tightly closed/sealed and properly stored. Additional laboratory best practices include performing inhalant testing on previously unopened tubes, treating inhalant samples as single-analysis-only and/or limited volume, minimizing sample handling or aliquoting, and offering qualitative/quantitative testing of inhalants in blood and/or urine for DUID (and PM) casework. As recreational use/misuse of N_2O continues to climb, forensic toxicologists should be aware of this growing trend and the challenges of detecting N_2O in biological specimens.

Disclosure

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

Conflict of Interest

2 - Salary/employment

S-34

One Driver and Three THC Cases Supporting the Three-Legged Stool Approach to Impairment

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Abstract

Introduction: Cannabis has been legal in Colorado for medicinal use since 2000 and recreational use since 2012. Despite public campaigns to limit marijuana-impaired driving, delta-9-tetrahydrocannibinol (THC) is the second most commonly reported substance by the Colorado Bureau of Investigation's toxicology laboratory behind ethanol. Among other impairments, THC has been shown to affect cognitive performance/skills, altered perception of distance/time, slowed reaction time, impaired balance/coordination, and decreased ability to perform divided attention tasks. In Colorado, a permissible inference level exists where juries may infer impairment if more than 5.0 ng/mL of THC in blood is present. Testimony can be provided to support or refute impairment. Ultimately, determinations of impairment should come using the three-legged stool concept reviewing toxicology results, driving behavior, and indications of impairment during interactions or performance of field sobriety tests (FSTs).

Objectives: This presentation will document the impairment and toxicology results from a 28-year-old woman who was involved in three separate crash/driving under the influence (DUI) investigations over a four-day period of time.

Methods: The samples were analyzed for ethanol and other volatiles using Headspace Gas Chromatography with dual flame ionization detector (HS/GC-FID). Samples were screened by a fourteen-panel ELISA. The cannabinoid screen target is 11-nor-9-carboxy-delta-9-tetrahydrocannabinol (THC-COOH) at 5.0 ng/mL. Cannabinoid confirmation is performed using liquid-liquid extraction with instrumental analysis via liquid chromatography tandem mass spectrometry (LC-MS/MS). The calibration range for THC and 11-hydroxy-delta-9-tetrahydrocannabinol (THC-OH) is 1.0-50 ng/mL and THC-COOH is 5.0-250 ng/mL. Cases can be reported as present less than the lowest calibrator if acceptance criteria is met. The measurement uncertainty for cannabinoid analysis is 19.2% with a 95% confidence interval.

Results: Offense 1 was a single vehicle crash onto private property. Offense 2, a DUI, occurred 52 hours later and was reported by a civilian in a parking lot. Offense 3 occurred 29 hours later and was a head-on-collision caused by the same driver. 'Low levels' of THC and metabolites were the only detected substances in each of the cases.

Offense Number/Type	Date	Time Between Stop and Draw (Minutes)	THC (ng/mL)	THC-OH (ng/mL)	THC-COOH ng/mL)
1 / Crash	9-24-24	52	4.8	1.9	44
2 / DUI	9-26-24	62	3.1	+<1.0	19
3 / Crash	9-27-24	66	2.9	ND	24

Discussion: In support of the three-legged stool concept, signs of poor driving and impairment were seen at each offense. Summarily the poor driving included running a stop sign, crashing into a fence and tree, side swiping a vehicle, and a head on collision caused by going the wrong way on a one-way street. At each offense, the officers noted lack of balance, inability to walk in a straight line, slurred speech, confusion, and the inability to retrieve important documents. In at least two offenses, FSTs including the walk and turn and one leg stand were either unable to be performed or stopped for safety. Only one case had lack of convergence performed and it was present. Additionally, the modified Romberg test was only performed once and demonstrated significant sway as well as body and eyelid tremors.

Toxicologists are always reminded to not determine impairment based solely on a drug concentration. In these three cases, the THC concentrations did not exceed the 5.0 ng/mL permissible inference existing in Colorado and could be described as 'low level' THC cases. However, in each case, the combination of: signs of impairment seen in FSTs/interactions, poor driving behavior, and toxicology results could support the conclusion that the subject was unable to safely operate a motor vehicle due to impairment from THC.

Disclosure

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

S-35

Rapid Sample Preparation and Screening of Gabapentin in Oral Fluid Using LDTD-MS/MS

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Abstract

Introduction: Gabapentin, a drug originally prescribed for the treatment of seizures, is now frequently used off-label for pain management. However, its use can cause side effects such as drowsiness and dizziness, which may impair driving and pose significant safety risks. Considering these issues, the National Safety Council's Alcohol, Drugs, and Impairment Division (NSC-ADID) has recently upgraded gabapentin's classification from a Tier II to a Tier I substance, adding it to the standard routine drug testing panel. For this purpose, NSC-ADID established a cutoff concentration of 50 ng/mL in oral fluid.

Objectives: The objective of this study was to develop a rapid sample preparation method for the extraction of gabapentin from the Quantisal device used for oral fluid collection. Additionally, in the context of rapid drug screening, a Laser Diode Thermal Desorption–Tandem Mass Spectrometry (LDTD-MS/MS) method was developed.

Methods: Oral fluid sample collection is performed by the Quantisal device, which is FDA 510(k) cleared for the collection of oral fluid for drug analysis. To prepare the samples, 10 µL of internal standard solution (gabapentin-d10 at 25000 ng/mL in acetonitrile) solution was added to a 12X75 mm borosilicate glass tube. Then, 125 µL of a spiked negative matrix diluted in Quantisal buffer (1:3) was added. The mixture was vortexed for 30 seconds at 1100 rpm. Subsequently, 500 µL of extraction solution and 1000 µL of dilution solution #1 were added. The mixture was mixed again for 30 seconds at 1100 rpm and centrifuged at 5000 rpm for 5 minutes. After centrifugation, 50 µL of the aqueous phase was transferred to a 0.5 mL Eppendorf tube and mixed with 150 µL of dilution solution #2. The final solution was mixed, and 5 µL aliquot was then spotted onto a standard LazWell plate and dried for 8 minutes at 40°C. LDTD-MS/MS method uses a maximum laser power of 65% with 6 seconds ramp and the carrier gas flow is set at 6 L/min. Analyses were carried out on an Axino Ion Source from Phytronix coupled with a Q5500 from Sciex operated in positive ionisation mode (APCI). Manual extraction of 12 samples requires 10.5 minutes, whereas automation would reduce this time. MS analysis is completed in under 10 seconds per sample.

Results: To validate the method, screening curves at 1X, 2X, and 5X the 50 ng/mL cut-off, plus QC samples at 0.5X and 2X, were prepared in drug-free saliva. The peak area ratio of targeted analyte against the internal standard (IS) ratio is used to normalize the signal.

For the inter-run precision/accuracy experiment, each fortified sample set is analyzed in triplicate each run. For the inter-run, linear calibration curves are performed, then accuracy and precision of back calculated concentration are evaluated for each level. Preliminary results show that accuracy, %Bias values between -3.3 to 2.5 % are obtained and the precision results are lower than 13.6% CV.

For each run (intra-run), a linear curve through zero with cutoff standards are used to evaluate the QCs. %CV lower than 3.3% are obtained from the cutoff standard. All QC-0.5X are detected as negative, and QC-2X detected as positive. For the matrix specificity test, different blank matrices (four) are analyzed as unknown samples. All matrices are detected as negative.

For the LDTD-MS/MS analysis, instead of studying the autosampler's stability, the wet stability (extracted solutions kept at 4°C) and dry stability (extracts on LazWell plate at room temperature) are evaluated. After the given stability time, standards and QCs are analyzed. The precision obtained for cutoff standards must be lower than 20%CV. Dry stability was confirmed for 1 hour at room temperature, while wet stability remains under investigation.

Discussion: The combination of LDTD with a QTrap 5500 mass spectrometer system from Sciex enables ultra-rapid (10 seconds per sample) screening of gabapentin in oral fluid. This method offers a rapid screening option for drug testing at roadside checkpoints using mobile crime labs equipped with mass spectrometers, as implemented by CEAEQ (Québec City) with TAGA unit and recently reported in Italy by Sciex and Forensic Lab Service (Soledad Poetto et al. (2024)).

Disclosure

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

Conflict of Interest

2) Salary / Consultant

S-36

Three-Year Review of Methamphetamine Findings in Suspected Impaired Driving Cases in Ontario, Canada

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Abstract

Introduction: Drug-impaired driving is an increasing public safety concern across Canada, particularly due to the demonstrated increase in use of recreational drugs such as methamphetamine. Methamphetamine is a central nervous system (CNS) stimulant drug that can impair an individual's driving ability in both the stimulant and crash phase. Despite the scientific consensus regarding the potential for methamphetamine to impair an individual's ability to operate a motor vehicle, there is relatively little information available regarding blood drug concentrations and associated observations of impairment in suspected impaired drivers.

Objectives: To further inform on the effects of methamphetamine on driving, the following were determined in authentic forensic casework: (i) the incidence of suspected impaired driving cases where methamphetamine and its metabolite amphetamine were detected alone, or in combination with other drugs, in blood and urine; (ii) observations reported by the arresting officer and Drug Recognition Evaluator (DRE) in suspected impaired driving cases where only methamphetamine and amphetamine were detected by toxicological testing.

Methods: Retrospective data analysis was performed to evaluate suspected impaired driving cases in which methamphetamine and amphetamine were detected alone, or in combination with other drugs, in blood and urine samples submitted to the Toxicology Section of the Centre of Forensic Sciences from 2021 to 2023. Drug testing was performed in all cases which was comprised of a broad general drug screen using liquid chromatography-quadrupole time-of-flight-mass spectrometry (LC-QTOF-MS) and targeted testing using liquid chromatograph-tandem mass spectrometry (LC-MS-MS). Observations of driving and subject behaviour were obtained from information provided in the submission including determinations made by the DRE at the time of subject evaluation.

Results: Of the 7,182 total suspected impaired driving cases, methamphetamine and amphetamine were detected in 569 of 2241 (25%) blood samples and 2266 of 4941 (46%) urine samples submitted. In 50 cases where methamphetamine and amphetamine were the only drug findings in blood, concentrations of methamphetamine and amphetamine ranged from 0.039 to 2.3 mg/L (mean 0.58 mg/L) and 0.007 to 0.29 mg/L (mean 0.085 mg/L) respectively. The average amphetamine-to-methamphetamine ratio in blood was 17%. There were 21 cases where methamphetamine and amphetamine were the only drug findings in urine. Driving observations in cases where only methamphetamine and amphetamine were detected in blood or urine included the driver being involved in a collision, the vehicle leaving the roadway, erratic driving, ignoring traffic signs, and the driver being asleep at the wheel. Observations of drug impairment reported by the DRE at the time of driver evaluation included abnormal speech patterns, poor balance and coordination, abnormal body movements, abnormal time perception, and the individual falling asleep. Other common observations reported by the DRE included eyelid tremors, bloodshot watery eyes, and droopy eyelids.

Discussion: Although typical indicators of acute methamphetamine administration were observed, the symptomology of methamphetamine's crash phase can mimic that of other drug classes such as opioids. Nonetheless, the DRE identified CNS stimulant drug impairment in the majority of cases. Unlike alcohol, the link between blood drug concentrations and impairment is not well established; however, the results provide insight into observations of impairment that may be associated with prior methamphetamine use and additional information to inform on the effects of methamphetamine on driving.

Disclosure

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

S-37

Hidden Dangers: Potential Unintended Fentanyl Exposure in DUID Drivers

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Abstract

Introduction: Fentanyl, in the form of counterfeit pills or powder, has been prevalent in the drug market since 2016 in Palm Beach County. Drug material composition can range from fentanyl alone to a combination with other drugs such as fentanyl analogs or other novel psychoactive substances (NPS). Fentanyl combined with other central nervous system depressants leads to an increased risk of cardiac and respiratory arrest. Drivers using fentanyl can demonstrate severe impairment up to and including an overdose event while operating a vehicle. These adverse effects represent a substantial peril to both the individual and traffic safety.

Objectives: This presentation summarizes four cases in which fentanyl was identified in blood specimens where the drivers stated they intended to use heroin, alprazolam, or cocaine. Signs and symptoms commonly observed with fentanyl alone and combined with other impairing substances will be highlighted as individual case reports are discussed.

Methods: All blood specimens underwent volatile analysis using HS-GC-FID/MS (Agilent 7890A/5975C). In 2023 drug screening included an eleven panel enzyme-linked immunosorbent assay (ELISA, Dynex DSX, Neogen kits). The ELISA fentanyl cutoff was 0.5 ng/mL. The ELISA screen was followed by a basic drug liquid-liquid extraction (LLE) with full scan GC-MS (Agilent 7890A/5975C) and all ELISA fentanyl positives were also screened by LC-MSMS (Sciex 5500+) for select NPS compounds. Blood cases submitted after October 2023 were screened using either an eleven panel or a three panel ELISA followed by targeted high-resolution mass spectrometry (Thermo Vanquish liquid chromatograph with a Thermo Q Exactive tandem mass spectrometer, LC-HRMS) that was validated for over 230 compounds with a fentanyl LOD of 0.5 ng/mL. Fentanyl was confirmed and quantified by LC-MSMS (Sciex 3200 Qtrap). The fentanyl confirmation LOD was 0.5 ng/mL, and the LOQ was 1 ng/mL. All other positive drug results were confirmed by GC-MS, LC-MSMS, or LC-HRMS. Confirmation testing did not include norfentanyl.

Results:

	Driver's Statement	Toxicology Results
Case 1	Purchased 2g of Cocaine	Ethanol at 0.128 g/dL, Fentanyl at 15 ng/mL, Xylazine, Tramadol at < 100 ng/mL
Case 2	Purchased Alprazolam (Capsules containing white powder)	Fentanyl at 6.6 ng/mL, Xylazine, Bromazolam, Carboxy-THC at < 5.0 ng/mL
Case 3	Purchased Heroin (Capsule containing white powder)	Ethanol at 0.130 g/dL, Fentanyl at 18 ng/mL, Carfentanil, Fluorofentanyl, N, N-dimethylpentylone, Norchlorcyclizine
Case 4	Snorted powder purchased as Heroin	Fentanyl at 27 ng/mL, Fluorofentanyl, Xylazine, Methadone at < 50 ng/mL

Discussion: The presence of fentanyl alone or in combination with other impairing substances resulted in serious adverse effects in drivers, where naloxone had to be administered in some cases to reverse overdose. Due to the severity of the incidents and the need for medical attention, no standardized field sobriety tasks were conducted. Observations of impairment noted came from law enforcement officers as they attempted to interact with the drivers before and after medical intervention. Drivers appeared to be confused, slow to respond, and unaware of what had occurred once they became responsive. Other common observations included: traffic crash (3/4), driver asleep at the wheel (3/4), unresponsiveness (4/4), and lethargic movements (4/4). All cases involved polysubstance use, and substances unknown to the user in the drugs purchased can have adverse interactions with the other drugs consumed. Limitations in the presented case series included that drug material was only tested in one case (one of two capsules), and other information on the perception of the drug consumed were from the users. Regardless, the presented cases demonstrate the significant risk that fentanyl poses to traffic safety, whether it was used knowingly or not. Hidden dangers to both the individual and the motoring public lie in the uncertainty in purity and composition of the drug supply and the adverse events that could occur including impaired driving, overdose, and death.

Disclosure

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

S-39

Determination of Synthetic Cannabinoids in MGG-Stained and Unstained Blood Smears: Innovations in Modern Toxicology

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Abstract

Introduction: Contemporary toxicology encompasses numerous research directions, one of which involves the search for alternative biological matrices that, in certain cases, may constitute the only available material in a given case. The complete blood count (CBC) is a fundamental test in clinical diagnostics. Despite the automation of procedures in diagnostic laboratories, manual blood smear analysis continues to play a crucial role in hematology.

Objectives: The present study investigated the applicability of manual blood smears, both unstained and stained with May-Grünwald-Giemsa (MGG), as a novel alternative biological matrix for the determination of xenobiotics, with synthetic cannabinoids (SCs) serving as representative analytes. Particular attention was given to the potential application of unstained smears for the analysis of blood traces on glass surfaces, with relevance to workplace drug testing, road traffic accidents, and the retrospective analysis of physical evidence archived by law enforcement agencies.

Methods: Analytical standards of selected almost 50 synthetic cannabinoids were added to K_2EDTA-stabilized whole blank blood, followed by the preparation of manual blood smears in accordance with protocols commonly used in medical diagnostic laboratories. Part of the smears were subsequently stained using the MGG technique. The developed analytical procedure involved transferring the matrix from the glass slide into a test tube using antistatic swabs with sponge tips, followed by liquid-liquid extraction under alkaline conditions using a mixture of *n*-hexane and ethyl acetate in the presence of isotope-labeled internal standards. Analyses were performed using ultra-high-performance liquid chromatography coupled with triple quadrupole mass spectrometry (UHPLC-QqQ-MS/MS) operated in multiple reaction monitoring (MRM) mode under positive electrospray ionization. For unstained smears, synthetic cannabinoids were quantified within a concentration range spanning from pg/mL to µg/mL. In addition to the reference total hemoglobin concentration typical for the general population, two additional levels were assessed: low (critical) and moderate. The method was validated and applied to assess the stability of SCs in blood smears stored at room temperature, which reflects typical storage conditions for such specimens.

Results: The developed method was validated and applied to determine the concentration ranges at which individual analytes could be reliably monitored. The findings demonstrated that unstained blood smears constitute a valuable alternative matrix for the detection of numerous xenobiotics—not only in cases of poisoning (toxic/lethal concentrations), but also for exposure assessment, enabling the detection of analytes at the ng/mL level, and in some instances, even at the pg/mL level (e.g. JWH-073, UR-144). Notably, effective xenobiotic detection was achieved despite substantial variations in total hemoglobin concentration. In contrast, the staining and

rinsing procedures associated with MGG-stained smears significantly limited the detectability of the analytes.

Discussion: The study results indicate that a minimal blood volume (less than 10 µL) is sufficient not only for qualitative but also for quantitative toxicological analysis, particularly in the case of unstained smears. Furthermore, the ability to detect substances at such low concentration levels underscores the need for careful interpretation of results, given the potential for contamination of glass surfaces—for example, prior to blood deposition—through earlier contact with fingertip sweat or saliva (e.g., from drinking), as well as during the collection, handling, and storage of samples as physical evidence in forensic investigations. Aside from the limitation associated with using blood collected in K_2EDTA tubes for manual smear preparation, both the developed method and the stability findings may prove valuable in toxicological analyses of native blood traces on glass surfaces.

Disclosure

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

Conflict of Interest

1) Grant: I (Karolina Nowak- presenting person) received the TIAFT Huestis & Smith International Travel Award to participate in the SOFT meeting.

S-40

Synthetic Cannabinoid Quantification on Infused Paper from Prisons by Liquid Chromatography Tandem Mass Spectrometry

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Abstract

Introduction: Synthetic cannabinoid (SC) use among prisoners is a major issue in several countries that contributes to health and safety concerns. The highly potent substances are sprayed onto paper and distributed to inmates through mailing services for discrete use. There is currently a lack of research dedicated to analyzing the concentration profiles of SC-infused papers.

Objectives: A method to screen and quantitate six SCs directly from paper was developed using Liquid Chromatography-Tandem Mass Spectrometry (LC-QQQ-MS) and applied to three authentic case samples from Cook County Department of Corrections. The research aimed to provide insight into the current infused prison paper market as well as providing quantitative analysis.

Methods: A LC-QQQ-MS method and extraction procedure was developed and validated per ASB guidelines for Forensic Toxicology and applied to three authentic samples from Cook County Department of Corrections. The study included a white 8 x 11 paper, smaller paper strips representing doses, and a smaller colored paper. Excluding the smaller strips of paper, samples were segmented into smaller pieces, extracted, and ran to develop heatmaps of SC concentrations across samples.

Results: All samples contained MDMB-4en-PINACA and its precursor MDMB-INACA. The SCs infused on the larger pieces of paper were highly variable (0.81mg/1.5 cm^2 paper - >10mg/1.5 cm^2 paper for MDMB-4en-PINACA and 50µg/1.5 cm^2 paper – 990µg/1.5 cm^2 paper for MDMB-INACA). Smaller strips of infused paper exhibited average parent and precursor concentrations of 520 µg/paper and 83 µg/paper respectively, representing doses that inmates take at a single time.

Discussion: The variable concentration profiles of MDMB-4en-PINACA and MDMB-INACA across infused paper samples make dosing by users in prisons dangerous as well as representative sampling by forensic analysts exceptionally difficult. This research represents a novel approach in testing seized prison materials with a high degree of sensitivity and accuracy, demonstrating the utility of LC-QQQ-MS for targeted SC analysis from paper.

Disclosure

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

S-41

Defining Identification Criteria for Routine LC-QTOF-MS Targeted Analysis

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Abstract

Introduction: Accurate identification criteria are crucial for reporting positive results in forensic toxicology, especially given the increasing prevalence of drugs of abuse, therapeutic agents, poisons, and novel psychoactive substances (NPS) in medico-legal cases. Liquid chromatography high-resolution mass spectrometry (HRMS) is widely used for comprehensive drug screening due to its untargeted acquisition capabilities, but challenges remain in analyzing untargeted data due to limited guidance on automated data processing and validation criteria. While general validation guidelines exist, specific HRMS parameters such as mass error, isotope ratios, and library scoring lack universally accepted standards. Variability in data processing software, peak detection algorithms, and spectral library searches further complicate standardization. To ensure consistency and reliability across laboratories, more precise and universally endorsed HRMS validation criteria are needed.

Objectives: This study aimed to establish the most efficient, sensitive, and specific criteria for detecting targeted analytes across all concentration levels. Additionally, it sought to define acceptable ranges for Combined Weight Score (CWS) parameters, including library scores, mass error, and isotope ratio. A key objective was also to determine effective approaches for validating these identification criteria in forensic settings. Once established, applicability studies were conducted to assess the reproducibility and reliability in authentic blood and urine samples.

Methods: The study developed a HRMS library using reference standards covering 1,038 compounds. Identification criteria parameters of mass error, retention time, isotope ratio, and library hit scoring were independently optimized through a comprehensive literature review and evaluation of authentic reference sample performance. A CWS was then optimized by testing multiple weight allocations for these parameters. A data matrix approach was applied to assess identification outcomes, categorizing results as true positives, false positives, false negatives, or true negatives based on user-defined thresholds. The optimized CWS model was then validated on proficiency test samples and 65 authentic case samples to ensure efficiency, sensitivity, and specificity.

To evaluate reproducibility, mass error, isotope ratio, and library scores were analyzed across 20 batches of blood and urine samples. Measurements at three concentration levels were assessed for statistical variation using ANOVA and Welch's t-tests. Correlation analyses, including Pearson and Spearman tests, explored relationships between key parameters and drug classes. Statistical analyses were performed using Python and visualized with Seaborn and Matplotlib, ensuring a rigorous approach to data interpretation.

To evaluate the applicability of the finalized identification criteria in routine forensic casework, over 400 authentic blood and urine samples were retrospectively analyzed. performance was assessed by comparing results to those obtained from validated in-house LC-MS/MS methods.

Results: Efficiency calculations for various weighting combinations and thresholds in blood and urine samples identified the optimal parameters: a threshold of 71 with weightings of 40% mass error, 40% library score, 15% retention time, and 5% isotope ratio. Applicability testing across 415 case samples yielded over 4,000 results for evaluation, demonstrating an efficiency and specificity of over 99% and sensitivity of ~90%. Statistical analyses revealed that library scores varied significantly with concentration, increasing at higher levels, while mass error remained stable. Isotope ratios exhibited greater variability at lower concentrations. Matrix comparisons showed that urine had lower library scores and CWS values than blood, with significant differences confirmed by t-tests. Drug class comparisons indicated only weak correlations with isotope ratios and mass error in urine, suggesting minimal impact on identification outcomes.

Discussion/Conclusion: This study underscores the importance of standardized identification criteria in HRMS for screening and confirmation. While mass error remained stable, allowing for stricter tolerances to reduce false positives, library scores improved with higher concentrations but were lower in urine, suggesting greater spectral variability due to matrix effects. Isotope ratios proved less reliable at lower concentrations and exhibited greater variability in urine, likely due to differences in ionization efficiency. Drug class had minimal impact on identification parameters, with only weak correlations observed for isotope ratios and mass error. A strong relationship between library scores and overall identification confidence highlights the critical role of spectral quality in analyte detection. While some compounds displayed outlier characteristics, refined reporting criteria allow for the identification of lower-confidence detections under specific conditions. These findings emphasize the need for robust thresholds and suggest further research into software-based optimization strategies to enhance long-term applicability and accuracy in forensic toxicology.

Disclosure

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

S-42

Analysis of Cannabinoids and Semi-synthetic Cannabinoids in Authentic Breastmilk Samples by Liquid Chromatography-Tandem Mass Spectrometry

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Abstract

Introduction: Marijuana (cannabis) is generally considered the most frequently misused substance during pregnancy. Although scarcely documented, the prevalence in the use of either medical or non-medical marijuana for relief of pregnancy-related symptoms is increasing, as well as the use of cannabis-related products containing cannabidiol (CBD) and semi-synthetic cannabinoids (SSCs). Δ^9-tetrahydrocannabinol (THC) and CBD are highly lipophilic substances and will readily pass into breastmilk upon ingestion. The solubility of THC and CBD in lipids poses significant analytical challenges in extracting and identifying these substances in breastmilk. Moreover, the recent and increased use of SSCs such as the Δ^9-THC structural isomer Δ^8-tetrahydrocannabinol (Δ^8-THC) has become popular among cannabis consumers and challenged the reliable detection of Δ^9-THC in forensic casework.

Objectives: To support the study and subsequent assessment of medical and non-medical marijuana and CBD use during pregnancy and postpartum, the aim of this study was to develop a new and sensitive assay utilizing liquid chromatography with tandem mass spectrometry (LC-MS/MS) to identify and quantitate cannabinoids in breastmilk.

Methods: The method was optimized to quantitate Δ^8-THC, Δ^9-THC, cannabigerol (CBG), CBD, and its precursor cannabidiolic acid (CBDA) and validated with the guidance of the American Academy of Forensic Sciences Standards Board (ASB) Standard 036. A variety of extraction protocols were evaluated prior to the performance of the validation. The assay was then used to analyze breastmilk samples (N=41) collected postpartum from female patients enrolled in a study assessing use behaviors of medical marijuana, non-medical marijuana, and CBD. The study protocol including the collection of breastmilk samples over various postpartum time points was approved by the Institution Review Board of the University of Florida (IRB-1#: 202202160; IRB-2#: 202300712).

Results: All analytes passed validation criteria. Calibration curves for all analytes had a range of 0.5-400 ng/mL. Δ^9-THC was quantitated in 12 samples (29.2%) with a concentration range of 1.0-291 ng/mL. Δ^8-THC was detected in one sample (2.4%) at 0.8 ng/mL, while CBD was identified in 2 samples at a concentration <LLOQ, and quantitated in only one sample (2.4%) at a concentration of 0.8 ng/mL. CBG was detected in 5 samples (12.1%) with a concentration range of 0.7-12.9 ng/mL, and at a concentration <LLOQ in 11 samples (Table 1).

Discussion: Removing the protein component from the breastmilk matrix is crucial in order to accurately quantitate the drug in the sample. Common protein crash procedures with organic solvents (e.g., methanol or acetonitrile) proved to be ineffective for this assay. The most efficient protein precipitation included sample pretreatment using a zinc sulfate solution. Besides THC and CBD, also emerging from the literature are the difficulties in the analysis of different cannabinoids such as CBG and SSCs in breastmilk due to the lack of information regarding their effects, redistribution, and accumulation in this biological matrix. The toxicological findings in this work demonstrate the capability of a newly developed assay to successfully detect and quantitate Δ^8-THC and other SSCs in authentic breastmilk specimens. Limitations of this work include a limited number of participants in the study and the poor stability of the processed samples that emerged during method development. This can be explained by the instability of cannabinoids in breastmilk specimens, storage and freeze/thaw cycles to which samples were exposed, and the potential degradation of certain compounds following the use of either acidic or zinc sulfate-based protein precipitation solutions.

The study presented here is a sensitive method for the analysis of cannabinoids in breastmilk to support, utilizing laboratory assay, the subsequent assessment of marijuana and CBD use during pregnancy and postpartum.

Table 1. Quantitative results of cannabinoids in the authentic breastmilk samples (N=41)

Compound Name		**Mean Concentration ± S.D.**	**Median Concentration**	**Range**
		(ng/mL)	**(ng/mL)**	**(ng/mL)**
Δ^9-THC	(n=12)	36.1 ± 81.2	12.3	1.0 - 291
CBG	(n=5)	3.6 ± 5.2	1.9	0.7 - 12.9
Δ^8-THC	(n=1)	0.8		
CBD	(n=1)	0.8		

Disclosure

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

Conflict of Interest

The authors have no conflicts of interest to declare.

S-43

Fast & Forensic: Rapid Detection & Quantitation of 30 + Emerging Novel Psychoactive Substances in Hair

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Abstract

Introduction: The continuous emergence of novel psychoactive substances (NPS) poses a significant challenge for forensic and clinical toxicologists, particularly due to their structural diversity, rapid turnover on the illicit drug market, and limited toxicological information. Hair analysis is a powerful matrix for retrospective drug exposure assessment and a great ally when it comes to monitoring NPS worldwide. However, the extraction of multi-class NPS in a single method remains an analytical challenge, especially in hair. As such, there is an urgent need for extraction techniques and analytical methods that are both rapid and capable of multi-class quantitation to keep pace with the evolving drug landscape.

Objectives: This study aimed to develop a fast, high-throughput method capable of detecting and quantifying over 30 emerging NPS (most reported on the market since 2024) across multiple chemical classes – including synthetic opioids, cathinones, cannabinoids, benzodiazepines, dissociatives, and hallucinogens – in hair samples. The goal is to support early monitoring efforts in forensic toxicology across different countries by targeting substances prioritized in the most recent Early Warning Systems.

Methods: Hair samples were decontaminated using sequential washes with deionized water, methanol, and dichloromethane. Once dried, they were weighed (20 mg) into 2 mL pre-filled metal bead mill tubes. Pulverization and extraction were carried out simultaneously in 1 mL of methanol, using a bead mill homogenizer with a program of 4.6 m/s for 10 cycles of 40 s, with a dwell time of 4 min with a total time of 43 min, where the temperature remained below 25°C. After centrifugation at 5000 rpm for 10 min, the supernatant was evaporated to dryness, and analytes were reconstituted in mobile phase's initial condition (0.1% formic acid in water: 0.1% formic acid in acetonitrile, 90:10, v:v), before clean up with nano PES-filter vials (0.2 µm). The detection of 31 NPS was performed using a Shimadzu 8030 LC-MS/MS system with a Kinetex C18 column (100 × 2.1 mm, 1.7 µm) under gradient elution, with a total run time of 10 min. The mass spectrometer operated in electrospray ionization (ESI) positive mode with multiple reaction monitoring (MRM) for quantification. Mobile phase A (MPA) consisted of 0.1% formic acid (FA) in ultra-high pure water (UHP) and mobile phase B (MPB) consisted of 0.1% FA in acetonitrile (ACN). As for internal standards, N-pyrrolidino-metonitazene-d3, 2 methylmethcathinone-d3, 5-Fluoro MDMB-PICA-d5 ketamine-d4 and flubromazepam-d4, in a concentration of 50 pg/mg.

Results: A fast and straightforward method for the analysis of 31 NPS in hair has been successfully validated according to the ANSI/ASB Standard 036 guidelines. Linearity was achieved with a linear curve model and a weighing of 1/x2 and not forced through origin, across a 6-day period with 7 calibrators: 5 (LLOQ), 10, 25, 50, 100, 250, 500 pg/mg. Precision was achieved with acceptable %CVs of <20% and accuracy remained within +-20%, with a low quality control (LQC) of 7.5 pg/mg, a Medium QC (MQC) of 75 pg/mg and a High QC (HQC) of 400 pg/mg. No

endogenous or exogenous interferences have been found, and matrix effects, extraction efficiency and process efficiency were all within acceptable ranges. No carryover was observed and samples remained stable for 48 h in the autosampler. As a proof of concept, hair samples obtained from electronic music festivals in Europe and Brazil have been tested.

Discussion: The present study represents an advancement in NPS detection in hair, offering speed, simplicity, and a monitoring tool to aid the shifting NPS landscape across the globe. This method is applicable to research groups, law enforcement, regulatory agencies and provides a great tool towards establishing NPS regional and global trends.

Disclosure

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

S-44

Community-Driven Surveillance of Emerging Drug Trends: 2024 Findings from Project EAGLE FANG in St. Louis County

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Abstract

Introduction: Project EAGLE FANG (Evidence And bioloGical Gathering for Laboratory Evaluation and Forensic Analysis of Novel druGs) is a collaborative drug testing initiative between the St. Louis County Department of Public Health and the St. Louis University Toxicology Laboratory (SSM SLUCare Physician Group, St. Louis, MO) supported by the CDC's Overdose to Action (OD2A): LOCAL grant. In 2024, the Toxicology Laboratory analyzed 187 anonymously submitted drug and paraphernalia samples from community-based organizations and the Department of Justice Services, offering a unique lens into the evolving local drug landscape.

Objectives: To enable timely identification and dissemination of substances present in the local drug supply to toxicology labs, public health entities, and the general public.

Methods: Drug materials and paraphernalia were voluntarily submitted by harm reduction agencies, the St. Louis County Medical Examiner, and behavioral health agencies. Submissions included capsules, pressed pills, paper products, baggies containing powders or residue, syringes, and residual solutions post-fentanyl test strip use. Each item was labeled with a unique identifier and suspected drug. Samples were dissolved in methanol and analyzed using liquid chromatography–quadrupole time-of-flight mass spectrometry and gas chromatography–mass spectrometry. Results were sent to participants within one week of submission to the lab. These results were posted in the lobbies of the participating harm reduction agencies and on an on-line dashboard (https://tinyurl.com/stlpef).

Results: Cocaine was the most frequently detected substance (57%, n=107), followed by fentanyl (53%, n=100) and diphenhydramine (52%, n=97). The most frequently identified cutting agents were diphenhydramine, caffeine, xylazine, medetomidine, lidocaine, acetaminophen, and levamisole, highlighting the complexity and variability of the unregulated drug supply. Notably, 60% of the top ten substances were cutting agents, underscoring the extent of drug adulteration. Poly-substance use was prevalent, with the most common combination being any opioid with fentanyl (49%). Xylazine, a veterinary sedative, was detected in 48% of samples and was frequently found with opioids and stimulants, raising concerns about its expanding role in the unregulated drug supply. Monthly trend analysis revealed shifts in drug prevalence. Cocaine positivity peaked in March (86%, n=12) and December (79%, n=15), while fentanyl remained consistently high, peaking in February (82%, n=18). Methamphetamine showed a notable spike in December (79%, n=15), and ketamine surged in October (47%, n=9). Novel synthetic opioids such as acetyl fentanyl (peaking at 68% in October) and metonitazene (25% in September, n=1) emerged sporadically. Synthetic benzodiazepines like bromazolam remained low but persistent, with a minor peak in July (18%, n=2). Other novel psychoactive substances rarely detected but showed isolated spikes, such as butylone in March (36%, n=5) and 2C-B (5%, n=1) in December.

Discussion: Results from the drug testing were sent in individual reports to area harm reduction agencies, behavioral health centers, medical toxicologists at area hospitals, and the Missouri Poison Control Center. For educational purposes, the results included a glossary that classified each drug, listed the associated overdose risks, and provided guidance on how to care for someone experiencing an overdose (e.g., administering naloxone). These results are used not only to inform public health and clinical professionals but also to educate people who use drugs about the potential harms associated with specific substances and combinations. The data supports harm reduction messaging and strategies, helping individuals make safer choices. Additionally, the findings guide clinicians in the medical management of substance-impaired patients by providing timely insights into the local drug supply and emerging toxicological threats.

This initiative not only helps ensure the laboratory's testing scope is aligned with emerging drug trends but also strengthens relationships with community partners by providing timely, actionable data. These findings inform harm reduction strategies, guide public health interventions, and foster trust between the laboratory and the populations it serves. Project EAGLE FANG exemplifies how forensic toxicology can bridge science and service to address the complexities of the modern drug crisis.

Disclosure

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

S-45

Is Hydrolysis Necessary in Urine Novel Psychoactive Substance Testing?

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Abstract

Introduction: Novel psychoactive substances (NPS) are synthetic drugs that are designed to mimic the effects of scheduled recreational drugs. NPS or "legal highs" are synthesized to circumvent laws banning more well-known recreational drugs and evade detection using traditional drug testing methods. NPS consist of synthetic compounds within the opioid, benzodiazepine, stimulant, and cannabinoid classes, as well as others. Many traditional opioid and benzodiazepine drugs of abuse (morphine, alprazolam) are glucuronidated in urine, a metabolic process that facilitates excretion of the drug from the body. Hydrolysis is frequently performed as a preanalytic step for these compounds to remove glucuronic acid and ensure all drug is measured when analyzing urine specimens. However, the utility of deglucuronidation in the context of NPS methods has not been explored.

Objectives: To determine the extent to which NPS in urine are affected by hydrolysis during sample preparation and how it affects drug positivity in patient specimens.

Methods: De-identified urine specimen remnants (n=30) were used for this study. All were selected based on previous positivity for ≥1 designer opioid analyte(s) using the Quest Diagnostics NPS panel. The panel includes NPS from 6 different drug classes (designer fentanyl analogs, designer benzodiazepines, designer opioids, designer stimulants, synthetic cannabinoids, and other illicit compounds). Results are reported semi-quantitatively by drug class. The designer opioid class consists of 20 total analytes and includes parent compounds as well as metabolites when commercially available as standards. A linear calibration curve was used, and cutoffs for analyte positivity ranged from 1 ng/mL to 10 ng/mL, depending on the designer opioid. In the original analysis, the extraction consisted of adding urine specimen, internal standard, and Kura B-One enzyme to a 96 well plate. The plate was incubated at room temperature for 30 minutes followed by a DPX tip-on-tip extraction and dilution. The reanalysis of specimens without hydrolysis involved replacing the enzyme with UTAK negative urine but otherwise following the same procedure. Specimens were analyzed on a SCIEX 6500+ LC/MS/MS System with Shimadzu Nexera LC system. Results were compared to determine the average fold decrease of calculated concentration by analyte and any changes in analyte positivity.

Results: When the specimens were analyzed without hydrolysis, 24 of the 30 (80%) remained positive for at least one of the 10 analytes within the designer opioid class with an average 56-fold decrease in calculated concentrations. Within these specimens, fold changes for calculated drug concentrations without hydrolysis were as follows:

pyrrolidino hydroxy-nitazene (160.92-fold lower in 19 specimens; only 2 (10%) specimens were positive without hydrolysis), hydroxy-nitazene (average 97.79-fold lower in 15 positive specimens; only 1 specimen [6%] was positive without hydrolysis), desethyl protonitazene (average 2.28-fold lower in 6 positive specimens; 5 [83%] were positive without hydrolysis), desethyl metonitazene (average 2.16-fold lower in 21 positive specimens; 17 [81%] were positive without hydrolysis), desethyl isotonitazene (1.74-fold lower in 1 specimen which was positive without hydrolysis), metonitazene

(average 1.27-fold lower in 14 positive specimens; 12 [86%] were positive without hydrolysis), Protonitazene (1.19-lower in 1 specimen, which was not positive without hydrolysis), pyrrolidino metonitazene (average 1.08-fold lower in 2 specimens which were positive without hydrolysis), pyrrolidino protonitazene (1.01-fold lower in 1 specimen which was positive without hydrolysis), and pyrrolidino etonitazene (0.74-fold lower in 1 specimen, which was positive without hydrolysis).

Discussion: When testing for designer opioids in urine specimens, hydrolysis is necessary to get an accurate representation of analyte presence and concentration in urine. If hydrolysis is not included in the sample preparation method, there may be a false decrease in reported positivity.

Conflict of Interest

2. Salary/Consultant.

S-46

Increasing the Ethanol Stop Limit Testing in Montana: Lessons Learned 1 Year Later

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Abstract

Introduction: Ethanol stop limit testing is a practice where additional drug testing does not occur if a case's blood alcohol concentration (BAC) exceeds a predetermined limit. Laboratories may institute stop limit testing due to constraints on resources like costs, staffing, caseload, and instrumentation, as well as turnaround time and local laws. However, this practice may lead to incomplete data of drug positivity.

Objectives: A retrospective analysis was conducted to evaluate the observed impacts of increasing a laboratory's ethanol stop limit testing from 0.100 g/dL to 0.160 g/dL one year after implementation.

Methods: Blood samples submitted for driving under the influence (DUI) investigations were analyzed at the Montana Forensic Science Division in Missoula. In January 2024, the laboratory initiated an increase in the ethanol stop limit from 0.100 g/dL to 0.160 g/dL after the creation of two toxicologist positions. All DUI blood cases (excluding those with Intoxilyzer results) were analyzed for volatiles via HS-GC-FID. Cases with ethanol results ≤ 0.159 g/dL were forwarded to drug screening, which includes a combination of ELISA immunoassay and an LC-MS/MS screen for 125 commonly encountered drugs. Cases involving driving fatalities received drug testing regardless of BAC result.

Results: This data contains all DUI cases tested from 2023 through 2024, including fatalities. In 2023, 3703 DUI blood samples were submitted and 1624 (44%) were tested for drugs. Of these, 60% were positive for alcohol only, 25% for drugs only, and 10% for alcohol and drugs. Approximately 59% of samples submitted were >0.100 g/dL. The top drugs were THC (40%), methamphetamine (24%), fentanyl (7%), and gabapentin (5%). The average turnaround time for all DUI offenses (including both ethanol only and drug testing) was 24 days, with 90% of cases completed in 46 days.

In 2024, 3852 DUI blood samples were submitted and 2370 (62%) were tested for drugs. Of these, 46% were positive for alcohol only, 25% for drugs only, and 21% for both alcohol and drugs. Approximately 40% of samples submitted were >0.159 g/dL The top drugs were THC (40%), methamphetamine (19%), fentanyl (5%), and gabapentin (5%). The average turnaround time for DUI offenses was 21 days, with 90% of cases completed in 34 days.

Comparing casework from 2023 to 2024 showed a significant increase in the number of cases involving THC alone (43%), THC combined with ethanol (106%) and THC combined with ethanol and other drugs (105%). In 2024, 588 non-fatal DUIs had a BAC between 0.101-0.159 g/dL with 185 (31%) having no additional drug findings. The most common drugs in this range were THC (234, 40%), benzoylecgonine (45, 8%), methamphetamine (34, 6%), and gabapentin (27, 5%).

Discussion: One year after implementing a higher ethanol stop limit, Montana DUI casework shows a large increase in cannabinoid positivity, especially in combination with alcohol. Review

of cases with BACs in the intermediate range of 0.101-0.159 g/dL revealed that benzoylecgonine surpassed methamphetamine as a common DUI finding. While the backlog remained relatively steady across both years, turnaround times decreased in 2024, possibly due to implementation of a paperless workflow in mid-2024.

Labs considering stop limit testing changes should consider several factors before deciding. Labs should review local laws to determine which BAC limits are relevant for stakeholders (i.e., aggravated DUI charges for BACs beyond 0.160 g/dL). Another consideration is staffing. Two additional toxicologist positions were created, but turnover challenges emerged with both toxicologists and support staff.

While adjusting or eliminating stop limit testing can establish more accurate drug positivity data, labs should consider the availability of resources prior to implementing changes. Evaluating factors like cost of materials, staffing, current and projected caseloads, instrumentation, and turnaround times are critical to determine feasibility.

Disclosure

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

S-47

A Tale of Two Propafenones: Utilizing a Fit-For-Purpose Method Validation and Method of Standard Addition for the Quantitative Analysis of Infrequently Encountered Analytes

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Abstract

Introduction: Propafenone is a Class 1C anti-arrhythmic drug used to control rapid and irregular heartbeats caused by atrial fibrillation (AFib) and paroxysmal supraventricular tachycardia (PSVT). Therapeutic serum concentrations typically range from 0.5-2.0 mg/L, with higher concentrations potentially leading to convulsions, hypotension, and central nervous system depression. Reports of fatalities involving propafenone toxicity are limited; therefore, monitoring this analyte for overdose potential is not common. However, at the Miami-Dade County Medical Examiner Department (MDME), two cases required propafenone quantitation. The first case, a suicide, prompted the development of a fit-for-purpose (FFP) quantitative method with little to no expectations of future use. The second case, certified as a natural death, showed a strong propafenone response in the blood drug screen, warranting additional quantitative analysis.

Objectives: The purpose of this presentation is to demonstrate the usefulness of a fit-for-purpose method validation with method of standard addition (MSA) analysis, to quantitate infrequently encountered analytes.

Methods: The FFP method was developed on a Shimadzu Nexera X2 ultra high-performance liquid chromatograph (UHPLC) coupled to a Shimadzu 8060 triple quadrupole-tandem mass spectrometer (QQQ-MS/MS). Separation was achieved by using a gradient elution on a Restek Raptor biphenyl column (50 x 2.1 mm, 2.7 µm, 90 Å) beginning with an 80:20 mobile phase ratio of 0.1% formic acid in water to acetonitrile. The QQQ-MS/MS operated in positive electrospray ionization (ESI) mode and data were acquired using multiple reaction monitoring (MRM), using three targeted transitions for propafenone. 0.500 mL of sample volume were prepared using solid phase extraction, and all analyses were conducted using MSA due to the limited scope of the FFP validation. The FFP method validation was conducted over four days and included evaluation of the calibration model, working range, limit of detection (LOD), limit of quantitation, precision, bias, carryover, and exogenous interferences.

Results: All validation criteria met predetermined specifications. The method demonstrated a working range of 0.10-5.0 mg/L using a quadratic calibration curve with a $1/x^2$-weighting. The LOD was administratively set at 0.05 mg/L based on experiments in nine different blood sources over two days. No carryover at 10 mg/L or exogenous interferences were observed. Precision and bias at concentrations of 0.20, 2.0, and 4.0 mg/L showed coefficients of variation (%CV) below 3% for both within-run and between-run assessments.

Case 1: A 22-year-old white female with diagnosed heart arrhythmia and a prescription for propafenone had been experiencing suicidal ideations. She was found unresponsive in her room and pronounced deceased at the emergency room. Quantitative analysis revealed propafenone concentrations of 8 mg/L in iliac vein blood, 20 mg/L in aorta blood, and a total of 1430 mg in gastric contents. Due to all sample concentrations initially being outside of the working range, dilutions were performed at a factor of x10, x50, and x1500, respectively.

Case 2: A 72-year-old white male was found choking at home and later pronounced deceased. The central blood propafenone concentration was 2.4 mg/L, slightly above the therapeutic range; the decedent was also prescribed venlafaxine, a common antidepressant. Literature review indicates a severe drug interaction between propafenone and venlafaxine due to propafenone's inhibition of the CYP2D6 enzyme, which alters venlafaxine metabolism. Concentrations of venlafaxine and its active metabolite, O-desmethylvenlafaxine, were 1.45 mg/L and 0.08 mg/L, respectively, suggesting elevated levels that may have contributed to the death and warrant further consideration.

Discussion: By applying a FFP validation and MSA analysis, reliable quantitative results were obtained for a rarely encountered analyte in two cases. This approach demonstrates a practical and efficient solution for postmortem forensic laboratories when full method validation is not practical or justified. It allows for case-specific quantitation while adhering to quality standards and can be seamlessly expanded into a fully validated procedure if additional cases arise.

Disclosure

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

S-48

Detection of Cannabinoids in Breath After Cannabis-infused Edible Consumption

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Abstract

Introduction: Currently there is no reliable measurement to determine if someone is driving under the influence of Δ^9-tetrahydrocannabinol (THC), the main psychoactive component of cannabis. Breath sampling is non-invasive and is already used to detect alcohol consumption roadside. THC has been collected from breath with multiple sampling devices, but studies published to date have been focused on cannabis inhalation, despite the multiple modes of recreational cannabis use. In particular, the detection of THC after cannabis-infused edibles consumption remains unexplored.

Objectives: The objective of this study is to determine if THC increases in breath after consumption of cannabis-infused edibles with multiple collection devices as a proof-of-concept and explore the trends of cannabinoid concentration after ingestion.

Methods: Breath was collected from 29 cannabis users before and at three time points (approximately 45, 90, and 180 min) after monitored edible consumption. Participants were requested to abstain from ingesting cannabis-infused products for 12 h and from inhaling cannabis products for 8 h prior to their session. Participants provided their own cannabis infused edible (5 – 100 mg THC). Both occasional and daily cannabis users were recruited, but usage profiles were not explored here. Breath was collected with two single-use devices – an impaction filtration device (n=23) and an exhaled breath condensation device (n=6). The impaction device was eluted by wetting the filter surfaces with 150 µL of solvent. Condensate was concentrated with lyophilization and reconstituted with 100 µL of solvent. Both solvents contained approximately 10 ng/g of deuterated internal standards. The identity and concentration of eleven cannabinoids and ten internal standards were determined with liquid chromatography tandem mass spectrometry using multiple reaction monitoring of two transitions for each cannabinoid.

Results: THC was detected in most breath samples, both before and after cannabis-infused edible ingestion, with both breath collection devices. THC was detected in breath samples from 27 of the 29 participants before edible ingestion, ranging from below the quantification limit (< 0.015 ng/device) to > 0.4 ng/device. THC was detected in all breath samples after cannabis use, although with variable trends. Of the 29 participants in this study, 19 showed a significant increase in THC concentration after edible ingestion (at one of the three post-use timepoints with subsequent decreases), 4 showed no change, and 6 showed a significant decrease in THC concentration after edible use. Six of the 29 participants had THC in their breath samples and no other cannabinoids that our method targeted. The remaining 23 participants had at least one of the following five non-THC cannabinoids: cannabidiol (CBD), cannabinol (CBN), cannabigerol (CBG), cannabichromene, and tetrahydrocannabinolic acid.

Discussion: THC was detected after cannabis consumption with both breath collection devices. Despite the limited post-use timepoints, 65 % of participants showed a significant increase in THC concentration after consumption and the increases are assumed to be from the observed cannabis-infused edible ingestion. The 6 participants with decreasing THC trends suggest that timepoints beyond 3 h should be investigated to ensure that the effect of edible ingestion is not

missed. When detected, CBN and CBG matched THC trends, but there were multiple instances where CBD and THC disagreed, suggesting differences in biological processing or clearance. These results show that further research into breath-based measurements and the determination of recent use with multiple modes of cannabis consumption is warranted. However, the high detection rate of THC in breath after 8 h of self-reported abstinence shows that identifying recent use from a single measurement is challenging from any matrix. Future work will focus on determining if THC's rate-of-change between two breath samples is a better measure of recent use than THC concentration in a single breath sample.

Disclosure

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

S-49

Stability of THC Concentrations in Breath Samples from Regular Cannabis Users after Overnight Abstinence from Cannabis Use

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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 tetrahydrocannabinol (THC) in breath increases immediately following cannabis use. While industry is developing devices for onsite THC collection or detection in breath, challenges remain to achieving a meaningful measurement that can be deployed in a variety of scenarios (e.g., workplace, roadside). While there are claims that THC is not detectable in breath after the recent use period associated with cannabis impairment (timescale of hours), small-scale published studies have often reported THC in breath upon arrival at the study site. If THC persists in breath beyond the impairment window, single timepoint measurements are not sufficient to determine recent use. Large studies with replicate breath samples are required to resolve whether multiple timepoints could be used instead to provide a reliable measurement.

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 uncertainty of measured THC concentrations in breath through the collection of replicate samples, which is necessary to investigate whether multiple timepoints can be used to determine recent use.

Methods: The study participants are regular cannabis users (e.g., daily or near daily use). Participants provided one blood sample and three breath samples at intervals of 5 min and 15 min on three separate days: an initial session (clinical setting) and two experimental sessions (mobile laboratory). Blood samples were collected by venipuncture and breath samples were collected with an impaction filter device with simultaneous measurements of breath volume and flow rate. At the experimental sessions, one additional blood sample and ten breath samples were collected at specified timepoints for two hours after cannabis use. Liquid chromatography-tandem mass spectrometry was used to positively identify and quantify THC in breath samples. The accuracy of the analytical method is within ±20% and the lower limit of quantitation (LLOQ) is 10 pg/g (approximately 3 pg/device). Only the breath samples collected before cannabis use will be described here.

Results: More than 70% of participants reported abstinence from 10 h to 16 h prior to the study session, while the remainder reported abstinence up to 60 h. Approximately 80% of the breath samples collected before cannabis use contained THC (positively identified); 20% were negative. THC concentrations as high as 200 pg/device were measured, but the majority of samples were below 50 pg/device. Fold changes, which are calculated by dividing a later measurement by the initial measurement, were calculated from breath samples collected from the same participant at the same session. More than 90% of comparisons had THC concentrations that increased or decreased by less than a factor of two, which we considered insignificant fold changes. Additionally, greater than 95% of comparisons had concentration changes that were below 20 pg/device.

Discussion: While this is an on-going study, approximately 150 breath samples have been collected from 17 regular cannabis users at multiple sessions after overnight abstinence. The majority of samples contained THC that was positively identified and quantified, demonstrating that a single positive THC breath sample does not indicate recent use. THC concentrations from breath samples from the same participant at the same session are stable based on fold changes and concentration changes. These results indicate that THC could be greater than zero beyond the window of impairment for regular cannabis users and that two timepoint measurements could potentially be a better indicator of recent cannabis use.

Disclosure

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

S-50

Assessing Real-Time Impairment Using the DRUID® App Following Alcohol and Cannabis Use in a Controlled Study.

Sumandeep Rana[1], Michael Milburn[2], Christopher Dale[3], Rohan Rana[1], William DeJong[2]

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Abstract

Introduction/Objectives: Over the past few years, several technologies have been developed to detect cognitive and psychomotor impairment due to substance use and other causes. Traditional toxicology tests detect presence, not performance, while Standardized Field Sobriety Tests (SFSTs) can be time-consuming, subjective, and logistically demanding. A need exists for a quick, mobile, and reliable screening tool to assess impairment in real-time. This IRB-approved investigation evaluated the DRUID® mobile application's ability to detect substance-induced impairment in a controlled study environment following alcohol and cannabis use. DRUID app uses video game-like tasks to assess reaction time, decision-making, divided attention, balance, hand-eye coordination, and time perception.

Methods: A total of 21 healthy adult participants between the ages of 23-65 years (8 females, 13 males) were recruited. Inclusion criteria included prior experience with cannabis and/or alcohol, absence of medical conditions, no experience with the DRUID app within the last one year, and a requirement to abstain from alcohol, cannabis, nicotine, and caffeine for at least 24 hours prior to the study. Participants self-selected into either the alcohol (n=10) or cannabis (n=11) portion of the study. Three subjects enrolled in both alcohol and cannabis portions. Each group had one control participant who did not consume either substance.

In the alcohol group (n=10), participants consumed 80-proof vodka ad libitum until they reached a breath alcohol concentration (BrAC) of 0.10% or higher, as confirmed using a calibrated breathalyzer. BrAC was measured at ten intervals, spaced 30 minutes apart, beginning after the target BrAC was reached. DRUID assessments were conducted at baseline (prior to drinking) and during each interval following consumption.

In the cannabis group (n=11), participants consumed cannabis ad libitum by smoking or vaping until they reached their desired level of intoxication. DRUID assessments were conducted before consumption to establish an individualized baseline, immediately after consuming the product, and then repeated at 30-minute intervals for ten testing rounds post-consumption. A Certified Drug Recognition Expert (DRE) conducted a full impairment evaluation immediately after the first post-consumption DRUID test.

For this study, a 5-point or greater increase from baseline on the DRUID scale was used as the threshold for significant impairment. This threshold was established based on prior DRUID research and Green Lab findings.

Results/Discussion: In the cannabis group, DRUID and DRE assessments aligned for 9 of 11 participants (82%) with an average DRUID score-difference of 12.3 (range 5.6 – 20.9) points from baseline at peak impairment. In the two cases where DRUID and DRE determinations did not match, DRE indicated impairment while the DRUID scores were 3.8 and 2.2 points over the individual baselines and did not meet the 5-point requirement for establishing a significantly impaired state.

Prior studies by Spindle et al. (2021), Karoly et al. (2022), and Zamarripa et al. (2025) have all demonstrated DRUID's sensitivity to cannabis-induced impairment.

Among the alcohol-consuming participants, 7 of 9 showed DRUID score increases of 5 points or more from the baseline, with an average increase of 18.0 points (range: 5.7–35.3). The remaining two participants had increases just below the cutoff, at 4.8 and 4.6 points. Further, the scores for alcohol participants tracked closely with BrAC. As BrAC declined over time, DRUID scores similarly decreased, with average scores returning to baseline when average BrAC dropped below 0.04. This suggests a relationship between DRUID performance and the level of alcohol-induced impairment. These findings are consistent with previously published research by Richman and May (2019) that showed DRUID scores rising proportionally with alcohol intoxication.

Conclusion

DRUID was able to detect impairment following both alcohol and cannabis use in a controlled setting, with strong alignment to DRE evaluations and BrAC, respectively. These results suggest DRUID as a viable rapid screening tool for real-time impairment detection across these two substances.

Disclosure

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

Conflict of Interest

I serve on the Advisory board of the company Impairment Science Inc. and the study involves the application of their impairment assessment app.

S-51

Rocky Mountain "High" THC Concentrations in Suspected Impaired Drivers in Colorado – A One-Year Analysis

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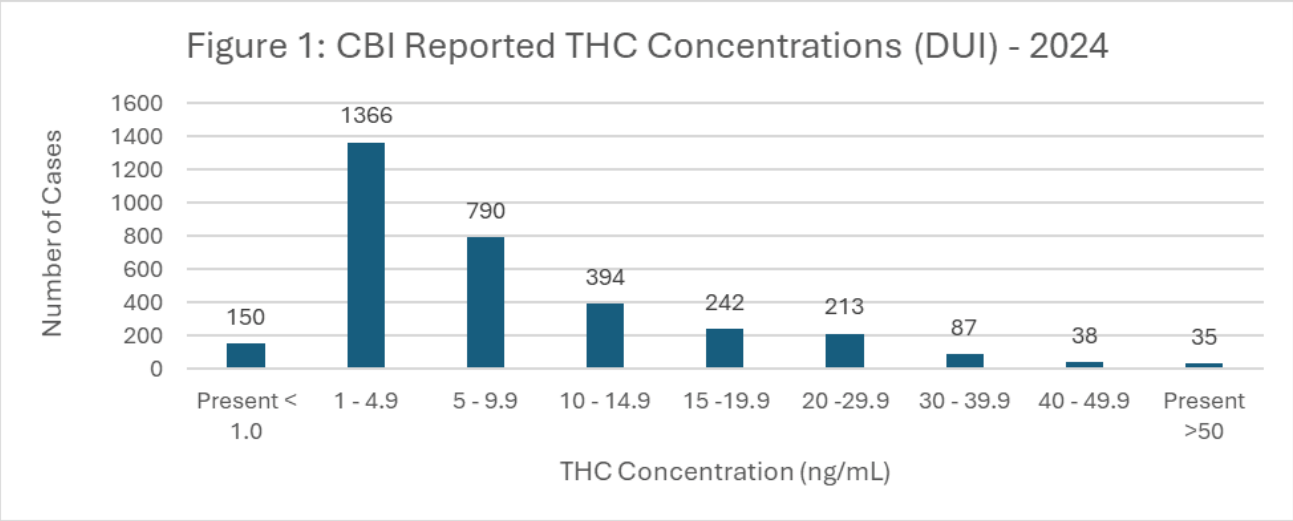
Abstract

Introduction: Colorado legalized Marijuana for recreational use over 10 years ago, in 2012, with the first recreational stores opening in January 2014. While Alcohol remains the most commonly detected substance among impaired drivers in the state of Colorado, THC is the second most commonly detected drug, and the two are often found in combination. Also of interest, high THC concentrations are routinely detected in casework as shown in Figure 1. The distribution of THC concentrations reported in Driving Under the Influence (DUI) casework in 2024 did not change significantly when compared to 2022 and 2023.

Objectives: This presentation aims to highlight the prevalence of elevated THC concentrations among suspected impaired drivers in Colorado in 2024. Additionally, cases with THC concentrations at 10, 30 and 50 ng/mL will be examined for poly-drug prevalence. Case studies with elevated THC concentrations will be presented.

Methods: All DUI cases submitted to the Colorado Bureau of Investigation (CBI) undergo Ethanol/Volatile analysis and a 15-panel drug screen. The drug screen is by Enzyme Linked Immunosorbent Assay (ELISA) and includes Cannabinoids (Cutoff – THC-COOH 5 ng/mL). Confirmation for Cannabinoids is performed via Liquid Chromatography Tandem Mass Spectrometry (LC/MS/MS) to include THC, THC-OH, and THC-COOH. The calibration range for THC and THC-OH is 1.0- 50 ng/mL and for THC-COOH is 5.0-250 ng/mL. In validation studies, 1:2, 1:5, and 1:10 dilutions were deemed acceptable for case samples for all Cannabinoids in order to report quantitative values beyond 50 ng/mL.

Results: In 2024, CBI completed 9,022 cases, of which 3,315 (36.7%) cases reported a THC concentration. The reported THC concentrations ranged from less than1.0 ng/mL – 124 ng/mL with a mean of 9.4 ng/mL. The distribution of cases across the calibration range is illustrated in Figure 1. In 1009 cases (30.4%) the reported THC concentration was greater than 10 ng/mL, and in 160 cases, the reported THC concentration was greater than 30 ng/mL. In 35 cases the THC concentration was greater than 50 ng/mL. An analysis was conducted to determine poly-drug trends at high THC concentrations, greater than 10 ng/mL, 30 ng/mL and 50 ng/mL.



Cases were categorized based on the reported THC concentration and, if applicable, other drugs detected in the case. The most common categories were Alcohol and THC, and THC only. In cases where THC was reported at a concentration greater than 10 ng/mL, 45.9% of cases were Alcohol and THC, while 36.9% were THC only. As the reported THC concentration increased, THC only cases became more prevalent. In cases where THC was reported at a concentration greater than 30 ng/mL, 46.3% were THC only, while 40.0% were Alcohol and THC. In the last category of cases, with THC greater than 50 ng/mL, 45.7% of cases were THC only and 42.9% were Alcohol and THC. Figure 2 below shows the categories of THC concentration and the percentage of cases falling into either the Alcohol and THC category or THC only category. In all three categories, Stimulants were seen in combinations with THC, and with and without Alcohol as the next most prevalent category.

Figure 2: Percentage of cases by THC concentration and Case Type

Category	THC > 10 (ng/mL)	THC > 30 (ng/mL)	THC > 50 (ng/mL)
Alcohol and THC	45.9%	40.0%	42.9%
THC Only	36.9%	46.3%	45.7%
Alcohol, THC and Stimulants	6.6%	2.5%	N/A
Cannabis and Stimulants	3.1%	3.1%	2.9%

Discussion: It is unknown whether the high THC concentrations seen in some Colorado drivers is consistent with other states or due to other factors. In 2024, the average time between the DUI stop and blood draw was 90 minutes, again consistent with past years. Additionally, despite increasing concentrations of THC, Alcohol continues to be commonly detected in conjunction with THC among drivers. Other drugs such as Stimulants are also seen in combination with THC despite increasing THC concentrations. Presented case studies will demonstrate drivers with elevated THC exhibiting different levels of impairment, highlighting the complexity of interpreting THC-related impairment in DUI casework.

Disclosure

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

S-52

Examination of High Alcohol Concentrations in Drug-Facilitated Sexual Assaults

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Abstract

Introduction: To the general public, drug-facilitated crime (DFC) cases specifically drug facilitated sexual assaults (DFSA) have allegedly been carried out by gamma-hydroxybutyrate (GHB) and flunitrazepam. However, ethanol (alcohol) is the most commonly associated drug with these cases due to its accessibility and general acceptance as safe because of its legal status.

The Houston Forensic Science Center (HFSC) receives approximately 3900 alcohol requests per year. Of these cases, 0.1% include DFSA investigations with significantly high blood alcohol concentrations (BAC). These results are rare for DFSA case types due to the time delay in specimen collection.

Objectives: The purpose of this study was to evaluate high blood alcohol concentration (> 0.200 g/100 mL) DFSA cases between January 2020 and December 2024. Case specific information was obtained from police reports and toxicological data.

Methods: DFSA data was compiled over a four-year period from January 2020 to December 2024. Cases with a BAC greater than 0.200 g/100 mL were identified (N=16), and case specific information was reviewed. Cases were initially analyzed for blood alcohol by gas chromatography flame ionization detection (GC-FID) followed by drug screening in urine with enzyme linked immunosorbent assay (ELISA) for common drugs of abuse (DOA) and/or gas chromatography-mass spectrometry (GC-MS) for other basic/neutral compounds and drug confirmation through liquid chromatography tandem mass spectrometry (LC-MS/MS). Due to the longer detection window for drugs of abuse and delays in specimen collection, drug screening and confirmatory analyses are performed in urine at HFSC for DFSA cases.

Results: Sixty-three percent of the high BAC cases included female complainants, and the other 37% were unidentified. Of the 63%, 6% were reported as black, 6% as Asian, 50% as other, and 38% were unidentified. Furthermore, 81% included alcohol and drug positives/presumptive positives. The 5 cases with the highest BAC were selected for discussion. Three of the 5 cases were female and drug positive. The average collection time for these high BAC cases was 4.8 hours. Based on this small sub-set of data (N=5), there appears to be no correlation between collection time and BAC ($r = 0.1797$).

Discussion: Case 1 involved a 41-year-old who was reportedly a victim of human trafficking. The complainant had a BAC of 0.324 g/100 mL and presumptive positives of amphetamines, benzodiazepines, and opioids. Case 2 included a 29-year-old female with a BAC of 0.297 g/100 mL and presumptive urine positive for benzoylecgonine. She was three months pregnant and could not recall her bus ride to where she was found. Case 3 was a 29-year-old Only Fans creator with a BAC of 0.482 g/100 mL with confirmed urine positives for alprazolam, alpha-hydroxyalprazolam, fentanyl, norfentanyl, and 11-nor-9-carboxy-delta-9-tetrahydrocannabinol (THC-COOH). She was found unconscious in her apartment. Case 4 involved a 37-year-old female who reported being forced into her apartment and assaulted. She had a BAC of 0.297 g/100 mL and presumptive urine

positives of amphetamines, benzodiazepines, and THC-COOH. Lastly, case 5 was a 47-year-old, gender unidentified, who reported being drugged and assaulted by their ex-spouse. The results were presumptive positive for THC-COOH in urine and had a BAC of 0.316 g/100 mL. Confirmation results are still pending for cases 4 and 5 but will be presented.

This study supports shorter collection times with higher BAC levels as the average overall collection time was 4.8 hours (N=16). Furthermore, it emphasizes the need to report DFSA cases as soon as possible to detect other impairing substances since 80% of the cases involved polydrug detection. Limitations of this study include lack of confirmation data for some of the drug screening results.

Disclosure

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

S-53

Assay Effective in Identifying Synthetic Urine Products - Potential Solution for Workplace and Clinical Drug Testing

Svante Vikingsson[1], Lauren Johann[1], Gabby DiEmma[1], Olivia Skirnick[1], Katherine Bollinger[1], Ronald R Flegel[2], Eugene D Hayes[2], Lisa S Davis[2], Ruth E Winecker[1]

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Abstract

Introduction: Using synthetic urine products to "beat" urine drug tests has become a business, with a wide range of commercial products available. The prevalence of synthetic urine use differs by testing purpose and population but has been reported to be 2-3%, similar to total drug positivity in some programs. Synthetic urine products are designed to register as authentic urine in common Specimen Validity Tests (SVT) including creatinine, pH, oxidants, and uric acid. The products can be identified by measuring panels of endogenous analytes by LC-MS/MS. However, LC-MS/MS methods are expensive as an initial test in workplace or clinical drug testing. Recently, Validity Diagnostics (Orlando, FL) introduced the True Urine Long and Short Duration Immunoassay SVTs, that identify authentic urine specimens by the presence of different urinary tract proteins. The assay can be operated using an automated analyzer and could potentially be used as an initial test to identify synthetic urine.

Objectives: To determine the sensitivity and specificity of the True Urine Long and Short Duration Assays by conducting an independent proof-of-concept study. A laboratory routinely using the assays was contracted for the analysis, but neither the laboratory or the manufacturer contributed to the study design, data evaluation or dissemination.

Methods: Fifteen synthetic urine products (purchased online in December 2024), 80 fresh deidentified urine specimens, and 16 older frozen specimens (four with pH 8.6-8.7 and four with pH 4.7-4.8) were included in the test (n=111). All samples were analyzed with the True Urine Long and Short Duration assays with the laboratory routine cutoffs. The synthetic urine specimens were verified by a qualitative LC-QTOF-MS assay measuring creatinine, theobromine, paraxanthine, and three unidentified chromatographic features previously found to be present in authentic urine (*m/z* 185, 229 and 265).

Results: While all synthetic urine samples contained creatinine, none contained theobromine, paraxanthine, or any of the chromatographic features, confirming them as synthetic urine products. The Long Duration assay correctly identified 14/15 synthetic urine products (93% sensitivity), as well as all fresh urine specimens (80/80) and 15/16 of the frozen ones (95/96, 99% specificity). The Short Duration assay correctly identified 8/15 synthetic urine products (53% sensitivity), as well as 78/79 fresh urine specimens and all frozen urine specimens (94/95, 99% specificity). The synthetic urine product incorrectly identified as authentic urine by the Long Duration Assay was also incorrectly identified as authentic urine by the Short Duration Assay. No result was obtained for one of the fresh urine specimens using the Short Duration Assay (clot detected flag).

Discussion: The True Urine assays, and especially the Long Duration assay, correctly identified synthetic urine products. The sensitivity was high enough to act as a deterrent to synthetic urine product use and high selectivity will minimize subsequent confirmation testing. When uric acid was

introduced as a potential synthetic urine initial test, manufacturers quickly pivoted to include uric acid in the products. However, urinary tract proteins are expected to be difficult and expensive to source and formulate making it more difficult for the manufacturers to overcome. In conclusion, the True Urine Long Duration Assay successfully identified synthetic urine products with high sensitivity and specificity, showing potential to serve as a synthetic urine initial test in combination with a mass spectrometry based confirmatory testing in clinical and workplace drug testing.

Ronald Flegel, Eugene Hayes, and Lisa Davis contributed to this work in their personal capacity. The views expressed do not necessarily represent those of the Substance Abuse and Mental Health Administration, the Department of Health and Human Services, or the United States government.

Disclosure

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

S-54

Investigating the *In Vitro* Metabolism of Several Brorphine-Based Novel Synthetic Opioids Using Human Liver Microsomes

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Abstract

Introduction: Novel synthetic opioids (NSO) containing the piperidine benzimidazolone scaffold (collectively referred to as "orphines") have begun to appear in illicit drug markets with an increasing frequency. Brorphine, the most well-known compound in the orphine class, was described as a potent synthetic opioid in a 2018 article.[i] It was associated with at least 21 fatal overdoses in the United States between April 2020 and June 2022, ultimately leading it to be listed as a schedule I drug by the Drug Enforcement Administration in 2023.[ii] As expected, after the scheduling of brorphine, other structural orphine analogs have been produced by illicit compound manufacturers and sold on both surface web and dark web marketplaces. These orphine analogs are an attempt to circumvent scheduling restrictions and be able to offer potent opioids that are technically legal to sell. N-propionitrile chlorphine, SR 17018, and 5,6-dichloro brorphine represent a few of the newer orphine analogs that have been identified in illicit markets.[iii]

Objectives: The goal of this study is to identify, using liquid chromatography and high-resolution mass spectrometry, probable phase I metabolites of the NSO orphine-type analogs N-propionitrile chlorphine, SR 17018, and 5,6-dichloro brorphine in pooled human liver microsomes (pHLMs).

Methods: For the metabolism studies, pHLMs were incubated with or without either N-propionitrile chlorphine, SR 17018, or 5,6-dichloro brorphine, each in triplicate, using the following protocol:

To a 1.5 mL Eppendorf tube containing 5 mL pHLM (20 mg/mL protein, previously aliquoted and stored at –80°C) was added 0.5 M phosphate buffer (79.8 mL, pH 7.5), 4.3 mL superoxide dismutase (20 units), and 1 mL drug substrate solution (1 mg/mL in MeOH). The mixture was vortexed and pre-incubated for 5 min at 37°C. Ten mL of an NADPH regeneration system (1.5 mL BioIVT K5000 product diluted with 3.5 mL deionized water to achieve the concentrations 1 mM NADP, 5 mM G6P, and 1 unit/mL G6PDH) was added to the pre-incubated reaction mixtures to achieve a final assay volume of 100 mL. Target drug concentration was 10 mg/mL. All samples were vortexed prior to incubation at 37°C for 60 min. Reactions were quenched with 100 mL ice cold acetonitrile, then 10 mL 10 M ammonium formate was added. Samples were vortexed and then centrifuged at 13,200 rpm for 15 min. The organic supernatant was analyzed using UPLC coupled to the electrospray ionization source of a Q Exactive Orbitrap mass spectrometer scanning for full MS and ddMS/MS over the *m/z* range 100-800. Metabolites were identified by their accurate *m/z* values and corresponding MS/MS spectra consistent with published metabolic pathways of brorphine in HLM.[iv]

Results: In this study, evidence was found supporting the formation of metabolites of the compounds tested via HLMs. These included, for instance, N-desalkylation, N-oxide formation, hydroxylation, or dihydroxylation, similar to what was observed for brorphine previously.[iii] With N-propionitrile chlorphine, evidence showed two distinct N-desalkylated metabolites. One of these resulting from the loss of the 1-(4-chlorophenyl)ethyl moiety, similar to the related loss observed in brorphine, and another having lost the propionitrile moiety instead.

Discussion: NSO brorphine analogs are becoming more prevalent in illicit drug marketplaces and have begun to show up in toxicological samples.[v] Identification of their primary metabolites will aid toxicologists in their casework and give them the tools they need to stay ahead of this growing area of opioid abuse.

[i] Kennedy N. M. *et al*. Optimization of a Series of Mu Opioid Receptor (MOR) Agonists with High G Protein Signaling Bias. *J. Med. Chem*. **2018**, *61*, 8895-8907.

[ii] Schedules of Controlled Substances: Placement of Brorphine in Schedule I, 21 CFR Part 1308 **2023**, https://www.federalregister.gov/documents/2023/03/06/2023-04364/schedules-of-controlled-substances-placement-of-brorphine-in-schedule-i

[iii] News: May 2025 –Emerging analogues of brorphine https://www.unodc.org/LSS/Announcement/Details/55d787ca-cec7-4204-90b0-68236305dcaa

[iv] Grafinger, K. E., Wilde, M., Otte, L., and Auwärter, V. Pharmacological and Metabolic Characterization of the Novel Synthetic Opioid Brorphine and its Detection in Routine Casework. Forensic Sci. Int. **2021**, 110989.

[v] NPS Discovery New Drug Monograph: N-propionitrile Chlorphine. https://www.cfsre.org/images/monographs/N-Propionitrile-Chlorphine-New-Drug-Monograph-NPS-Discovery.pdf

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 Cayman Chemical Company and also hold shares of company stock.

S-55

Case Series Involving N-Isopropyl Butylone: A Novel Synthetic Cathinone Implicated in Fatalities

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Abstract

Introduction: The international control of drugs creates significant shifts in novel psychoactive substance (NPS) markets, especially with relation to synthetic stimulants and their use. Eutylone was placed under control in 2021 which resulted in the emergence and proliferation of *N,N*-dimethylpentylone which was under control in 2024. *N*-Isopropyl butylone emerged in August 2024 and has since competed for positivity with *N,N*-dimethylpentylone. *N*-Isopropyl butylone is a positional isomer of *N,N*-dimethylpentylone, a substance classified as a Schedule I drug in the United States due to its isomeric relationship to *N*-ethyl pentylone. *N*-Isopropyl butylone presents risks to public health comparable to other synthetic cathinone stimulants due to increasing involvement in fatal and non-fatal overdoses and possible adverse effects including hallucinations, hyperthermia, arrythmias, and serotonin syndrome.

Objectives: This study sought to develop an analytical framework for the identification and quantitation of *N*-isopropyl butylone with application to authentic medicolegal death investigation cases to provide reference concentration ranges of this new and emerging drug.

Methods: Toxicology specimens suspected of containing *N*-isopropyl butylone were comprehensively screened and reflexed for quantitative confirmation. Screening analysis was conducted using a Sciex X500R liquid chromatograph quadrupole time-of-flight mass spectrometer (LC-QTOF-MS) and quantitation was performed via standard addition using a Waters Xevo TQ-S Micro liquid chromatograph tandem quadrupole mass spectrometer (LC-QQQ-MS). The quantitative range was assessed from 1.0 to 100 ng/mL and standard addition was performed using up-spike concentrations of 1.0, 10, and 100 ng/mL. *N*-Ethyl pentylone-D5 was used as the internal standard. Samples (0.5 mL) were prepared using a single-step basic liquid-liquid extraction. Chromatographic separation of *N*-isopropyl butylone from all known isomers was achieved using an Agilent InfinityLab Poroshell C-18 120 (3.0x150mm, 2.7 µm) analytical column using gradient elution. Mobile phase compositions were 5 mM ammonium formate in water (pH 3) and 0.1% formic acid in acetonitrile. The flow rate was 0.4 mL/min. Injection volume was 5 µL. The method was validated prior to use. Specimens were subjected to the method and case information was collected, when available.

Results: *N*-Isopropyl butylone was confirmed in 15 postmortem cases and one antemortem case collected between August and November 2024. Cases originated from Florida (88%), South Carolina (6%) and Connecticut (6%). Individuals ranged from 28 to 57 years and were primarily male (69%). Case histories were provided for most cases. Causes of death were primarily attributed to multi-drug toxicity and blunt-force trauma. Individuals in this case series were struck by moving vehicles (20%) or found with altered mental status and/or unresponsive after apparent drug use (75%). *N*-Isopropyl butylone was confirmed in all specimens with a median blood concentration of 51 ng/mL (mean: 210±370, range: 1-1400 ng/mL). In one case, *N*-isopropyl butylone was

quantitated in blood, vitreous fluid, and urine, with concentrations of 18, 17, and 2200 ng/mL, respectively. *N*-Isopropyl butylone was detected alongside other synthetic cathinones (e.g., alpha-PiHP, alpha-PiHpP) in 50% of cases, fentanyl (75%), traditional stimulants such as cocaine and methamphetamine (63%), ethanol (31%) and other NPS such as bromazolam (19%) and MDMB-4en-PINACA (13%).

Discussion: This validated assay was successfully implemented for the quantitation of *N*-isopropyl butylone in biological specimens. It was determined to be a contributing factor to death in this case series and poses considerable consequences for health and safety of drug users. *N*-Isopropyl butylone is the most recent synthetic cathinone to emerge and proliferate on the illicit drug market and based on previous trends in synthetic cathinone prevalence, represents a significant shift in the national NPS stimulant market.

Disclosure

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

S-56

Extended Release, Extended Risk: A Fatal Case of Bupropion and Trazodone Toxicity

M. Elizabeth Zaney, Nicholas Barna, Jennifer Gonyea, Marissa Finkelstein, Diane Moore

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Abstract

Introduction: This case report involves a 25-year-old female with history of sickle cell beta-thalassemia disease, anxiety, depression and remote suicide attempt via amitriptyline ingestion. On the day of the terminal events, she had been discharged from an involuntary three-day psychiatric hospitalization, initiated after she threatened to overdose using her trazodone medication. When she returned home, she experienced continued social stressor(s) and proceeded to intentionally ingest an excess of her prescribed medications (50 mg immediate-release trazodone and 300 mg extended-release bupropion XL) to commit suicide. During transport to the hospital, she vomited approximately ten tablets and was noted by fire rescue personnel to be drowsy and to have stated that she "wanted to die". At the scene, police recovered three empty bottles of bupropion XL and one empty bottle of trazodone. Clinical findings at the emergency department included respiratory failure, metabolic and respiratory acidosis, and seizures. She was intubated, became hypotensive, and was admitted to the intensive care unit. Despite supportive care, she suffered cardiac arrest and died two days later. Autopsy revealed approximately 100 mL of thick, brown liquid and numerous white, partially digested tablets. The small intestine contained approximately 200 mL of thick, tan fluid from which one, predominantly digested, white tablet was identified. The colon and rectum contained soft, dark green-brown stool from which six, predominantly digested, white pills/tablets were recovered in the cecum.

Objectives: To demonstrate how postmortem drug concentrations vary over time in a delayed fatal overdose, highlighting the impact of drug class and pharmaceutical formulation (immediate vs. extended release) on distribution and absorption in biological specimens.

Methods: Toxicological screening included a comprehensive drug screen for the presence of volatile substances (ethanol, acetone, isopropanol, and methanol), illicit substances, prescription medications, and over-the-counter drugs. Quantitation of bupropion, hydroxybupropion, trazodone, and chlorophenylpiperazine (mCPP), was performed utilizing solid phase extraction followed by analysis on an ultra-high-performance liquid chromatograph coupled to a Shimadzu 8060 triple quadrupole tandem mass spectrometer.

Results:

Specimen	Bupropion	Hydroxy bupropion	Trazodone	mCPP
Admission Blood 12/27/24 11:45	0.15 mg/L	0.25 mg/L	7.4 mg/L	1.2 mg/L
Iliac Blood	40 mg/L	31 mg/L	0.85 mg/L	0.93 mg/L
Heart Blood	44 mg/L	25 mg/L	0.63 mg/L	1.1 mg/L
Brain	28 mg/Kg	78 mg/Kg	1.1 mg/Kg	9.6 mg/Kg
Liver	Detected	153 mg/Kg	1.2 mg/Kg	14 mg/Kg
Gastric	544 mg total	Detected	10 mg total	1.8 mg total
Small Intestine	4.1 mg total	16 mg total	0.67 mg total	1.1 mg total

Note: Date and time of death was 12/29/24 at 14:20; Autopsy specimens were collected on 12/30/24 at 11:44.

Other substances detected included sertraline, clonazepam, nordiazepam, hydroxyzine, and multiple hospital-administrated medications (lorazepam, midazolam, levetiracetam, propofol, lidocaine, atropine, and etomidate). Acetone was detected at 0.002 g/dL in admission blood and serum (diagnostic of ketosis); reflexive ocular fluid analysis revealed interval development of marked hypernatremia (sodium concentration greater than 180 mmol/L).

Discussion: Upon review of the medical records, the decedent was not treated with activated charcoal or gastric lavage. The evidence indicates that ongoing absorption of extended-release bupropion occurred during the hospital stay, resulting in clinical deterioration and dramatically elevated postmortem concentrations. In contrast, trazodone concentrations declined as expected. The combined toxic effects of bupropion, trazodone, and other possibly co-ingested substances ultimately led to multiorgan failure and death. Of note, postmortem microscopic analysis also revealed an associated vaso-occlusive crisis. The cause of death was certified as “Acute Combined Drug Toxicity Including Trazodone and Bupropion”, and the manner of death was ruled “Suicide”. This case underscores the potential need for aggressive early intervention in toxic ingestion cases involving extended-release drug formulations.

Disclosure

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

S-57

Quantitative Analysis and Postmortem Redistribution Evaluation of Select NPS Benzodiazepines in Postmortem Casework

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Abstract

Introduction: Since 2011, Novel Psychoactive Substances (NPS) have become increasingly prevalent in postmortem casework at the Miami-Dade County Medical Examiner (MDME). While fentanyl analogues and NPS stimulants are the most frequently encountered, NPS benzodiazepines have been detected in postmortem casework since 2017. These substances are structural analogues of conventional benzodiazepines; but vary in potency and pharmacological effects. The toxicological significance of NPS benzodiazepines in postmortem cases remains unclear; as a result, their contribution to death must be evaluated in the context of each case. To improve the interpretation of these findings, a quantitative method was developed and fully validated to ANSI/ASB Standard 036 for etizolam, bromazolam, flualprazolam, and flubromazolam in postmortem specimens. In addition to analyzing peripheral blood, any available central blood, liver tissue, and brain tissue collected in these cases were also analyzed to assess for postmortem redistribution (PMR).

Objectives: The purpose of this presentation is to assess PMR of select NPS benzodiazepines by analyzing authentic case specimens of liver tissue, brain tissue, central blood, and peripheral blood.

Methods: A Shimadzu Nexera X2 ultra high-performance liquid chromatograph (UHPLC) coupled to a Shimadzu 8060 triple quadrupole tandem mass spectrometer (QQQ-MS/MS) was utilized for the quantitation of etizolam, bromazolam, flualprazolam, and flubromazolam. Separation was achieved using a gradient elution on a Restek Raptor C18 column (50 x 2.1 mm, 2.7 µm, 90 Å) beginning with a 70:30 mobile phase ratio of 0.1% formic acid in water to acetonitrile. The QQQ-MS/MS was operated in positive electrospray ionization (ESI) mode. Data acquisition employed multiple reaction monitoring (MRM) with three targeted transitions per analyte. Quantitation was performed over a concentration range of 2 -100 ng/mL, with a limit of detection administratively set at 1 ng/mL. All specimens were prepared using solid phase extraction (SPE).

Results: A total of 174 specimens from 81 cases were analyzed, including 68 peripheral bloods, 69 central bloods, 18 liver tissues, 16 brain tissues, and 3 antemortem bloods. Among the cases, 83 positive identifications were made between May 2019 and May 2025: 24 etizolam, 44 bromazolam, 14 flualprazolam, 1 flubromazolam. PMR was assessed using both central blood to peripheral blood (C/P) and liver to peripheral blood (L/P) concentration ratios, when specimen availability allowed. For cases with antemortem blood, a PMR factor (F) was also calculated, following the method described in McIntyre (2016). Concentration mean, median, and range for all specimens are summarized in the table below. C/P ratios were only calculated for etizolam, bromazolam, and flualprazolam due to specimen availability; similarly, L/P ratios were determined only for etizolam and bromazolam. The average C/P and L/P ratios for each analyte are also shown below.

Specimen		Etizolam	Bromazolam	Flualprazolam	Flubromazolam
Peripheral Blood (ng/mL)	Mean	69.17 (n=23)	46.45 (n=35)	5.65 (n=12)	N/A
	Median	12.57	31.78	5.74	
	Range	2.33-386.97	4.88-169	2.21-9.00	
Central Blood (ng/mL)	Mean	72.03 (n=22)	50.07 (n=35)	6.05 (n=12)	N/A
	Median	13.12	31.32	6.50	
	Range	2.6-400.19	4.48-175	2.72-10.61	
Liver (ng/g)	Mean	143.65 (n=3)	249.10 (n=12)	32.28 (n=1)	218.15 (n=1)
	Median	122.37	119.09		
	Range	65.53-243.05	23.85-1007.75		
Brain (ng/g)	Mean	32.67 (n=2)	115.50 (n=11)	20.67 (n=1)	74.74 (n=1)
	Median	32.67	59.46		
	Range	20.69-44.66	14.22-374.94		

Table 1 – Concentration values of all analytes

Average Ratio	Etizolam	Bromazolam	Flualprazolam
C/P	1.09	0.91	1.18
L/P	5.61	4.76	-

Table 2 – Average C/P and L/P ratios

Discussion: Published data suggest that the average C/P and L/P ratios observed for these NPS benzodiazepines indicate minimal PMR. This finding is consistent with previous research on both NPS benzodiazepines and related triazolobenzodiazepines, such as alprazolam. Given the relatively small differences between central and peripheral blood concentrations, quantitating in central blood may serve as a viable alternative in cases where peripheral blood is unavailable.

Disclosure

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

S-58

Gamma Hydroxybutyrate: What Does the Concentration Mean? Review of Ante-mortem and Postmortem Casework from 2020 – 2025.

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Abstract

Introduction: Gamma hydroxybutyrate (GHB) is an endogenous molecule, a prescription drug, an illicit drug, and can be formed both ante-mortem and postmortem, making interpretation difficult especially in postmortem cases. As an endogenous molecule, GHB acts as a neuromodulator with primary activity at the gamma- aminobutyrate B ($GABA_B$) receptor; it is also a minor metabolite of GABA. Clinically, GHB was first used in the 1960s as an adjunct to anesthesia but was unpredictable regarding its duration of effect, likely due to its steep dose response curve. GHB is now used clinically to treat narcolepsy and alcohol withdrawal syndrome. As an illicit drug, GHB can be made from its precursors, gamma butyrolactone (GBL), and 1,4-butanediol (1,4-BD), both are industrial solvents and pro-drugs of GHB. GHB can be elevated in blood samples collected in sodium citrate tubes, and GHB can increase postmortem during the decomposition process.

Objectives: To review 401 cases sent to the lab that were tested for GHB, either as directed testing or as part of a drug panel. Casework consisted of both ante-mortem and postmortem samples. The cases were comprised of; twenty-five positive postmortem cases, twenty four bloods and 1 urine, out of thirty-one total postmortem cases targeted for testing of GHB (77%), and five positive ante-mortem cases, four urine, and one blood out of 371 total cases (1.3%) tested in a drug facilitated assault drug panel. A selection of these cases will be presented.

Methods: Blood and urine specimens are shipped to the lab, and blood is in tubes containing sodium fluoride and EDTA as preservative and anticoagulant. Currently, blood and urine samples are extracted using a cold methanol extraction containing internal standard with detection by liquid chromatography paired with triple quadrupole mass spectrometry (LC-MS/MS). The lower limit of detection (LOD), lower limit of quantitation (LLOQ) and upper limit of quantitation (ULOQ) are 2.5, 5.0 and 100 mcg/mL, respectively. Prior to December 2024, GHB testing was performed using solid phase extraction columns and gas chromatography mass spectrometry (GC/MS) detection. The LOD, LLOQ, and ULOQ were 4.0, 5.0 and 100 mcg/mL, respectively. Methods were validated using internal protocols based on ABFT requirements, and AAFS/ASB guidelines, and standards.

Results: The overall results for the postmortem blood and one urine and ante-mortem urine and one blood are listed in Table 1.

Number of Specimens (n)	Median mcg/mL	Mean mcg/mL	Range or Value mcg/mL
Postmortem Blood – all collection sites, n=24	78	215.9	6.0 to 1668
Postmortem Blood – Femoral/Peripheral n=10	76.1	196.2	9.9 to 560
Postmortem Blood – Cardiac n=6	136.4	134	9.1 to 271
Postmortem Blood – Central n=3	513	736.8	29.4 to 1668
Postmortem Blood – Unknown n=5	77.3	81.2	19.2 to 169
Postmortem Urine n=1	NA	NA	> 100 (value for single case)
Ante-mortem Blood n=1	NA	NA	6 (value for single case)
Ante-mortem Urine n=4	8.1	70.8	7.5 to 248

NA = Not available

For blood and urine cases (15) in which additional drugs were tested, the most common drugs found with GHB were methamphetamine and amphetamine, 67%.

Discussion: The lowest median GHB blood concentration occurred in the femoral/peripheral blood, the cardiac median was higher and the central median was the largest. The cases in which GHB use was suspected were not always the cases with the highest GHB concentration. Review of the literature shows various cut offs proposed for postmortem blood and urine GHB to differentiate between endogenous and exogenous GHB. The proposed postmortem cut offs range from 20 to 50 mcg/mL in blood and 10 to 30 mcg/mL in urine. There were 7 postmortem blood samples with a GHB of < 20 mcg/mL, 2 cases < 30 mcg/mL, and no cases between 31 and 50 mcg/mL. Interpretation of GHB in postmortem blood and urine can be problematic. A detailed history and the ability to test additional specimens, such as urine and vitreous fluid, may be necessary to help differentiate between endogenous production and exogenous ingestion, considering that urine and vitreous fluid are less susceptible to postmortem GHB production.

Disclosure

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

S-59

When Routine Toxicology is Negative: The Critical Role of Seized Drug Analysis in a Medicolegal Death Investigation Involving Vaping

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Abstract

Introduction: Novel psychoactive substances are often difficult to detect due to new formulations being introduced as legal alternatives to other regulated substances. Meanwhile, their increased potencies can challenge conventional limits of detection. This presentation describes a unique postmortem investigation where collaborative efforts identified the substances of interest and aided in determining the cause and manner of death.

Objectives: To emphasize the importance of working with seized drug partners to identify novel psychoactive substances in forensic toxicology casework.

Case History: A 22-year-old male was found deceased in bed with frothy edematous fluid emanating from his mouth and nares. The evening prior, he had purchased and used a new vaping product. The internal postmortem examination revealed extensive pulmonary congestion and edema with microscopic evidence of acute bronchiolitis. Edema and mixed inflammation were also found along cartilage and non-cartilage-lined airways, but diagnostic PCR was negative for SARS-CoV-2 infection. No other gross or microscopic pathologic abnormalities were noted.

Methods: Multiple vaping devices and a small liquid container were collected at the scene and submitted to the United States Criminal Investigation Laboratory for chemical analysis. The liquid samples were analyzed by GC-MS, GC-IR, LC-TOF/MS, along with a targeted LC-MS/MS panel for nitazenes. The residue from the vaping device was analyzed by GC-MS, GC-IR, and LC-TOF/MS. Postmortem toxicology specimens were collected at the time of autopsy and submitted to the Division of Forensic Toxicology. A routine analysis was performed, including ethanol and other volatiles, a drugs of abuse immunoassay screen, and separate LC-QTOF/MS panels for expanded drug screening and synthetic cannabinoids. Follow-up toxicology testing was conducted by the Center for Forensic Science Research and Education (CFSRE) using LC-QTOF/MS and LC-MS/MS.

Results: The routine toxicology results were negative for any volatiles or substances within the current scope of testing. However, analysis of vaping materials yielded the presence of CHO-4'Me-5'Br-FUBOXPYRA and protonitazene. Toxicology testing at CFSRE revealed these substances in blood and urine as well. The forensic pathologist attributed the cause of death to acute protonitazene and CHO-4'Me-5'Br-FUBOXPYRA intoxication, and the manner was accidental.

Discussion: CHO-4'Me-5'Br-FUBOXPYRA (CH-FUBBMPDORA) is a synthetic cannabinoid first identified in the U.S. in 2023 in seized materials. The case herein appears to be the first toxicological detection of this analyte in humans. Recent literature describes CHO-4'Me-5'Br-FUBOXPYRA as having limited cannabimimetic activity at both cannabinoid receptors. While opioids such as

fentanyl have been described in combination with synthetic cannabinoids in seized drug products, this is the first report of a nitazene and new generation synthetic cannabinoid detected in a vaping product and in toxicology specimens. This investigation illustrates the need for laboratories to maintain a contemporary scope of novel psychoactive substances with low limits of detection, particularly for potent substances like nitazenes. It also emphasizes the importance of collaboration between toxicology and seized drug laboratories in medicolegal investigations, especially when case history supports a drug-related death, but initial toxicology results are negative.

Disclosure

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

S-60

The Cost of Clarity: Death After Participation in a Heart Protocol Ceremony Involving MDMA and Ketamine

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Abstract

Introduction: In recent years, the use of psychoactive substances such as methylenedioxymethamphetamine (MDMA) and ketamine in mental health and wellness practices has increased, raising concerns about safety when adverse outcomes occur. This case report describes the death of a 51-year-old woman who participated in a wellness retreat aimed to provide clarity and relief from depression, anxiety, suicidal ideation, addiction, and stress. Her regimen included a gastrointestinal cleanse followed two days later by a guided psychedelic "heart protocol", during which MDMA and ketamine were administered by a licensed psychiatrist. She was monitored for twenty hours following the ceremony. One day later, the decedent entered a rooftop sauna and was found deceased the following day.

Objectives: To illustrate the importance of postmortem toxicology in elucidating the underlying physiological mechanisms leading to a complex drug-related death.

Methods: A comprehensive medicolegal investigation was conducted to include a forensic autopsy and comprehensive toxicology testing for the presence of volatiles, over-the-counter substances, prescription medications, and illicit drugs. The investigation also included a review of emails and text messages between the decedent and the administering psychiatrist, as well as the instructional materials detailing the substances and procedures used during the ceremony. Witness statements and documentation from the wellness center were also examined to verify environmental conditions, the decedent's activities during the retreat, and the timeline of events.

Results: The autopsy revealed pulmonary congestion, nephrosclerosis, and adhesions within the pleural cavities. Focal discoloration of the left hypothalamus was noted, along with evidence of a previously implanted cardiac device. External findings included diffuse skin slippage, drying, and early decomposition. Toxicology testing revealed the following:

Specimen Source	MDMA	MDA	Ketamine	Norketamine	DMT
Iliac Vein Blood	0.312 mg/L	0.335 mg/L	0.052 mg/L	0.079 mg/L	Not Detected
Heart Blood	Detected	Detected	Detected	Detected	Not Detected
Gastric contents	Detected	Detected	Detected	Detected	Detected

The concentration of MDMA, methylenedioxyamphetamine (MDA, a primary metabolite of MDMA), ketamine, and norketamine are, individually, not consistent with reported concentrations associated with fatal intoxications. Ketamine and MDMA were also identified in the gastric contents along with N,N-dimethyltryptamine. Vitreous fluid chemistry confirmed significant electrolyte abnormalities consistent with hyponatremic dehydration: low sodium (Na = 128 mmol/L), elevated creatinine (2.3 mg/dL), and elevated urea nitrogen (24 mg/dL).

Discussion: MDMA is known to impair heat dissipation via norepinephrine-mediated vasoconstriction, which can result in hyperthermia even in temperate environments. Its effect on vasopressin secretion can also promote water retention, increasing the risk of hyponatremia. Ketamine, a dissociative anesthetic, can intensify these effects by further impairing the body's ability to respond to thermal and electrolyte stress. The circumstances suggest that the pre-existing dehydration from the gastrointestinal cleanse, compounded by the physiologic effects of MDMA and ketamine, was critically worsened by confinement in the sauna. These factors together likely triggered a fatal electrolyte imbalance and cardiac arrhythmia.

Notably, DMT was detected in the gastric contents (not quantitated) but was not identified in the blood (limit of detection is 1 ng/mL). DMT is a hallucinogenic tryptamine known to cause intense visual and auditory disturbances, along with profound changes in mood and sense of self. It may also induce cardiovascular effects such as elevated heart rate and blood pressure. DMT is the primary psychoactive component of *ayahuasca*, a traditional Amazonian brew used ceremoniously, which the treating psychiatrist mentions in various podcasts and YouTube videos. Although neither *ayahuasca* nor DMT was specifically mentioned in the official protocol related to this fatality, documents did refer to the possible inclusion of a hallucinogen during the recovery phase. The absence of DMT in the blood may reflect limited systemic absorption, particularly if consumed orally, or its short half-life and rapid metabolism in the liver and gastrointestinal tract.

Based on the integration of autopsy findings, toxicology results, and the investigation, the cause of death was determined to be hyponatremic dehydration due to confinement in sauna and use of MDMA and ketamine. DMT was not considered a contributing factor due to its absence in the blood. The decedent's voluntary participation, informed consent, and the absence of evidence of intent to harm support classification of the death as accidental, in accordance with guidelines from the National Association of Medical Examiners.

Disclosure

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

P-001

Non-Invasive Detection of Psychoactive Substances in Breath Samples: Development of a Breath Collection Device

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Abstract

Introduction: Psychoactive substances pose public health and safety problems worldwide. Accurate detection of these substances is crucial for forensic toxicology. Traditional biological samples like blood, urine, oral fluid, sweat, and hair have limitations in collection feasibility, detection windows, and invasiveness. Breath analysis has emerged as a promising non-invasive alternative for psychoactive substance detection. Exhaled aerosol microparticles contain non-volatile compounds originating from the alveolar region of the lungs and offer a readily available and minimally intrusive sample for substance detection.

Objectives: In this study, we developed and validated a breath collection (BC) device for detecting psychoactive substances in exhaled breath.

Methods: Breath, blood, and urine samples were collected from 20 individuals with a history of substance use from an addiction treatment center in Türkiye. Breath samples were collected using a BC device to capture nonvolatile substances through filters. The filters were eluted with solvent. Blood and urine samples were analyzed using solid-phase extraction (SPE). All samples were analyzed using liquid chromatography-tandem mass spectrometry (LC-MS/MS). Method validation assessed linearity, accuracy, precision, recovery, LOQ, stability, and matrix effects per ANSI/ASB Standard 036.

Results: The method showed high selectivity, linearity ($r>0.99$), and sensitivity, with LOQ from 0.4 to 48.1 pg/filter for 24 analytes. Stability studies confirmed that the psychoactive substances remained stable on the filters for 7 days under various conditions. At least one psychoactive substance was detected in 95% of the breath samples (n=19). Methamphetamine was the most commonly used substance (90%; n=18) and was detected in both the blood and breath in 16 cases. In two cases, Methamphetamine was positive in breath and urine, but negative in blood. The median Methamphetamine concentration in exhalation samples was 78.08±104.4 pg/filter. Additionally, Benzoylecgonine (n=2), Morphine (n=1), 6-MAM (n=1), MDA (n=2), Amphetamine (n=13), Cocaine (n=2), MDMA (n=3), THC (n=1), Acetylcodeine (n=1), Gabapentine (n=3), Pregabaline (n=6), and THC-A (n=4) were detected in the breath.The Analytical Greenness calculator (AGREE) yielded a score of 0.55, indicating moderate alignment with green analytical chemistry principles.

Discussion: This study presented the first successful development of a BC device for the detection of psychoactive substances in breath samples in Türkiye. It provides a noninvasive and rapid alternative to traditional matrices, with substantial green chemistry alignment. These findings support the integration of breath analysis as a green and reliable alternative in forensic toxicology.

Keywords

Breath analysis, Psychoactive substances, Non-invasive sampling, Green chemistry, LC-MS/MS

P-002

Forensic Toxicology Data-Independent Analysis Screening Using Xevo™ MRT Mass Spectrometer Routine Parts-per-Billion (ppb) Mass Accuracy

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Abstract

Introduction: Laboratories are frequently required to perform broad screening techniques on complex biological matrices, to identify drug substances, and other toxicants in a quick and efficient timeframe. As well as accurate results and rapid turnaround times, laboratories are constantly under pressure to seek options that are cost effective and environmentally sustainable. Broadband data-independent analysis (DIA) and targeted post-acquisition data processing has fast become the preferred method for screening of forensic samples. Currently, the analytical strategy uses high-resolution mass spectrometry to facilitate the collection of an unbiased dataset, providing a complete profile of the sample, including precursor and fragment ion information.

Objectives: The study demonstrates a significant advancement in Data-Independent Acquisition (DIA) specificity by leveraging parts-per-billion (ppb) mass accuracy, achieved using the benchtop hybrid quadrupole multi-reflecting time-of-flight mass spectrometer (MS), the Xevo MRT MS. Both a commercial standard and authentic urine samples were analyzed to detect illicit and non-illicit drugs, along with their metabolites. The results highlight the method's superior selectivity, enhanced mass accuracy, operational efficiency, cost-effectiveness, and environmental sustainability.

Methods: Broadband DIA (MSE) was performed using the Xevo MRT MS. Data were acquired using the Forensic Toxicology Screening solution in ESI+ ionization mode, with an acquisition rate of 10 Hz and a collision energy ramp of 10-40 eV. Samples were analyzed using reversed phase chromatography comprising a 15-minute gradient elution with a C18 column, maintained at 50°C. Data were acquired for the commercial standard, Waters system suitability test (SST) mixture, at 25ng/mL and ten anonymized authentic urine samples (diluted 1:10). Compound identification was based on three criteria: alignment of retention time within ±0.35min of reference value, detection of the precursor mass, and the presence of at least one diagnostic fragment ion. Analytical data were cross-referenced with the established Waters toxicology library, which includes retention time and exact mass for both the precursor and fragment ions for more than 2000 toxicologically relevant compounds and metabolites.

Results/Discussion: All detections in the Waters SST mix and authentic urine samples, were identified using enhanced stringent data processing tolerances. An RMS error of 466ppb was achieved for the SST mix where the mass accuracy tolerance was set to ±2ppm (precursor ion mass) and ±0.2mDa (fragment ion mass). All expected components together with their expected fragment ions were detected.

The sub-ppm mass measurement and high specificity attained for the SST mix, provided high confidence in analyte identifications made in the ten anonymized authentic urine samples, which included illicit and non-illicit drugs and their metabolites. An RMS error of 571ppb was achieved for 160 precursor ion identifications from the ten urine samples and attained a detection rate of 94% within 1ppm. An RMS error of 613ppb (0.083mDa) was achieved for 92 fragment ions, obtained from 23 randomly selected identifications, a detection rate of 96% within 1ppm (0.2mDa) was attained.

Conclusions: This study demonstrates the enhanced specificity and selectivity of Xevo MRT MS, for the detection of analytes in complex biological matrices. The step-change in performance significantly improves analytical efficiency by reducing false positives, thereby reducing the number of samples requiring confirmatory analysis. This reduces operational costs and contributes to improved environmental sustainability. The application of ppb-level mass accuracy, combined with game changing stringent data processing tolerances, increases confidence in the detection of both illicit and non-illicit drug substances based on retention time, precursor and diagnostic fragment ions. These advancements collectively streamline toxicological workflows and elevate the reliability of compound identification.

Conflict of Interest

I am an active employee of Waters Corporation, Wilmslow, UK.

P-003

Detection of Tier I and Tier II Drugs in Oral Fluid Samples Collected in Missouri DUI Cases

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Abstract

Introduction: The prevalence of drug-impaired driving behavior has increased in the United States, complicating the efforts of law enforcement. Situations in which drivers are impaired through legal or illegal drug use create challenging scenarios to prosecute and remove these drivers from the road. The toxicology community has done well to monitor drug trends and establish a recommended tier system based on impairment risk and prevalence of certain drugs. Guidance for oral fluid testing has also been established, making it a valuable matrix in toxicology testing for law enforcement. This abstract will highlight how oral fluid was used to test for Tier I and Tier II drugs in oral fluid samples collected in Missouri DUI cases.

Objectives: The objective of this study was to measure the prevalence of Tier I and Tier II drugs in DUI casework collected in Missouri. Additionally, the range of quantitative results measured for delta-9-THC was calculated.

Methods: Drug recognition experts (DRE's) across the state of Missouri collected oral fluid samples for laboratory testing during a non-evidentiary trial with Forensic Fluids Laboratories. All DUI samples (n=56) were tested using ELISA and LC-MS/MS. Quantitative results were collected from LC-MS/MS testing. The detection rate of Tier I, Tier II, and Tier I + Tier II drugs were evaluated. The range of concentrations of delta-9-THC were also calculated.

Results: Across all 56 cases, 39 different drugs or metabolites were measured in oral fluid. 21 were classified as Tier I, 12 were classified as Tier II, and 6 were unclassified (mainly metabolites of a Tier I drug). 36 of the cases were positive for only Tier I drug(s). 17 cases were positive for both Tier I and Tier II drug(s). 1 case was positive for only Tier II drug(s). Cases positive for Delta-9-THC (n = 40) ranged from 0.25 ng/mL to >500 ng/mL, with a median concentration of 31.3 ng/mL. 64% of the cases were positive for more than one drug.

Discussion: State laws in Missouri allow for the use of oral fluid testing in driving under the influence cases. Little to no testing has been done using oral fluid in this state, so this information serves to highlight the ability of oral fluid to measure various Tier I and Tier II substances and assist law enforcement in the state with their DUI traffic safety initiatives.

P-004

Leveraging QTRAP Technology for Ultra-Sensitive Quantitation of 11-nor-9-carboxy-THC (THC-COOH) in Hair

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Abstract

Introduction: With over 192 million consumers worldwide in 2018, cannabis is the most commonly abused recreational drug. Detection of its use can be performed in several biological matrices such as blood, urine, oral fluid and hair. In recent years, hair testing has gained considerable attention as a quicker and less invasive means of monitoring cannabis use. The use of this matrix for drug testing benefits from larger detection window, ease of sampling, observed collection and difficulty of sample adulteration.

Objectives: In this study a highly sensitive LC-MS/MS method was developed, using MRM^3 acquisition, for the quantification of THC-COOH in hair. MRM^3 acquisition is a unique QTRAP scanning function and improves selectivity and specificity through the acquisition of second-generation fragment ions. This improved selectivity has been shown to reduce background noise, resulting in lower detection limits in complex matrices.

Methods: Blank hair samples were prepared using a dilute and shoot sample preparation procedure. Liquid chromatography was performed using a SCIEX ExionLC AC system and the mass spectrometry analysis was performed on a SCIEX 7500 system with negative mode electrospray ionization. The acquisition mode was MRM^3. The Guided MRM^3 automated compound optimization feature in the SCIEX OS software was used to optimize the MRM^3 acquisition parameters. Three replicates of each calibrator was injected to build a data analysis processing method.

Results: A series of 6 spiked calibrator hair samples ranging from 0.2 to 10 pg/mg were injected in triplicate. The use of the MRM^3 workflow on the 7500 system enabled accurate quantitation of THC-COOH down to 0.2 pg/mg, starting from 50 mg of hair. The MRM^3 workflow demonstrated excellent linearity of the generated regression curves, with an R^2 value > 0.99 across the calibration range. The results of the MRM^3 experiment at the lowest calibrator level (0.2pg/mg) showed excellent precision and accuracy, demonstrating the quantitative performance of the method.

Discussion: A highly sensitive workflow for the detection of THC-COOH in hair was developed using the 7500 system. The use of the MRM^3 workflow was shown to increase selectivity by significantly reducing background and matrix interference, resulting in a LOQ of 0.2 pg/mg.

P-005

Assessing the Long-Term Stability of Synthetic Cannabinoids in Human Blood by LC-QQQ-MS

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Abstract

Introduction: Synthetic cannabinoids, classified as novel psychoactive substances (NPS), have emerged as significant drugs of abuse, differing from delta-9-tetrahydrocannabinol (THC) in both chemical structure and pharmacological effects. Their increasing prevalence presents challenges for forensic toxicology laboratories in detection, quantification, and interpretation of results.

Objectives: This research focused on three primary objectives: (1) to develop a liquid chromatography-tandem mass spectrometry (LC-QQQ-MS) method capable of quantifying the synthetic cannabinoids MDMB-PINACA, MDMB-PICA, MDMB-BINACA, and MMB-PICA; (2) to validate the method in accordance with the AAFS Standards Board (ASB) and American National Standards Institute (ANSI) *Standard Practices for Method Validation in Forensic Toxicology*; and (3) to evaluate the stability of these parent analytes in human whole blood preserved with sodium fluoride and potassium oxalate under refrigerated conditions (4°C) over a 60-day period.

Methods: A targeted LC-QQQ-MS method was developed and optimized for the detection and quantification of the four synthetic cannabinoids. Validation parameters including accuracy, precision, limit of detection, limit of quantification, and matrix effects were assessed according to ASB/ANSI guidelines. For the stability study, fortified drug-free human whole blood samples were stored at 4°C and analyzed at multiple time points (day 0, 1, 2, 7, 14, 28, 40, and 60) to monitor concentration changes over time. Analyte stability was defined as remaining within 20% of the initial concentration.

Results: An accurate and precise method was successfully validated for MMB-PICA, MDMB-PICA, MDMB-BINACA, and MDMB-PINACA. Stability testing revealed that all analytes, except MMB-PICA, remained stable for up to 40 days under refrigerated conditions. MMB-PICA demonstrated rapid degradation, with a 90% decrease in concentration observed within the first 24 hours and a significant decline by day 60. MDMB-PICA and MDMB-PINACA remained stable through day 40, followed by significant declines by day 60. MDMB-BINACA was the only analyte that maintained stability above the 80% threshold through the entire 60-day period.

Discussion: The findings demonstrate notable differences in the stability of structurally similar synthetic cannabinoids under typical forensic sample storage conditions. The rapid degradation of MMB-PICA highlights the importance of timely analysis following sample collection. The validated method developed in this study provides a reliable tool for forensic toxicology laboratories, while the stability data offers guidance for the handling, storage, and interpretation of blood samples containing synthetic cannabinoids. Future research should investigate alternative storage conditions or the use of other preservatives to enhance analyte stability over extended periods. Additionally, further testing of other synthetic cannabinoids featuring “MMB” head groups could help determine whether the instability observed with MMB-PICA is structurally driven. Including a broader range of synthetic cannabinoids in future studies would help strengthen comparative stability assessments.

P-006

Distribution of Fentanyl in Postmortem Brain Specimens

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Abstract

Introduction: The Drug Enforcement Agency reports more than 107,000 Americans died from a drug overdose in 2023, with approximately 70% attributed to fentanyl. Fentanyl is a potent agonist of mu-opioid receptors, which are located primarily in the central nervous system. Brain has utility for postmortem fentanyl testing because it is relatively easy to obtain during autopsy, is available in ample quantities, is amenable to sample preparation, and is less susceptible to decomposition than centrally located organs. However, interpretating fentanyl results in brain is complicated by highly localized sites of action, potential uneven distribution and limited literature.

Objectives: Determine if there is homogenous fentanyl distribution in seven different regions of the brain - basal ganglia, cerebellum, medulla, midbrain, occipital lobe, thalamus, and pons.

Methods: Cases with a fentanyl-positive urine drug screen at autopsy were considered for study inclusion. Cases were excluded if they were homicides, decomposed, involved head trauma, and/or died in a hospital. Brain tissue was homogenized as a 1:4 dilution by weight with Milli-Q 18.2 MΩ deionized water using an Omni International bead ruptor (Kennesaw, GA). Triplicates of each sample were subjected to solid phase extraction using UCT Clean Screen® DAU columns and analyzed by an Agilent 1290 Infinity Series liquid chromograph coupled to an Agilent 6460 tandem mass spectrometer (Santa Clara, CA). Separation was achieved using gradient elution with an Agilent Poroshell 120 phenyl hexyl column (2.1 x 100 mm, 2.7 µm) and matching guard column. Mobile phases consisted of (A) 0.1% formic acid in deionized water and (B) 0.1% formic acid in acetonitrile. Concentrations were determined with a blood-based calibration curve (range 0.5-100 ng/mL). The limits of detection and quantitation in brain homogenates were 0.5 ng/g and 1.5 ng/g, respectively.

Within and between-subject average brain concentrations were evaluated for the seven brain regions. For between-subject evaluations, concentrations were normalized to occipital lobe, as occipital lobe is commonly collected in our agency. Statistical analysis was performed using IBM SPSS Statistics Version 29.0.1.0 (Chicago, IL).

Results: In 100 cases analyzed, 86 were positive for fentanyl. When present, fentanyl was found in all seven brain regions; however, fentanyl was not homogeneously distributed, as within-subject concentrations were significantly different in the seven brain regions using one-way ANOVA ($p<0.05$). Fentanyl concentrations were highest in the basal ganglia when normalized to occipital lobe (43% of positive cases). There were no cases where the medulla had the highest fentanyl concentrations when normalized to the occipital lobe and few cases where the pons (1.1%) or cerebellum (3.4%) had the highest fentanyl concentrations. In seven cases, average fentanyl concentrations by brain region were between the limit of detection and quantitation. Between-subject concentration difference between brain regions was statistically significant using one-way ANOVA ($p<0.05$).

Discussion: Despite varying concentrations within and between brain regions, any of the seven brain portions included in this study could have utility for postmortem toxicology testing. The brain sampling locations were selected based on their concentration of mu-opioid receptors and the ability to obtain a large enough sample size for testing. While some regions had lower fentanyl concentrations (medulla, pons, and cerebellum), fentanyl was still detectable in these regions. The basal ganglia is located in the center of the brain and controls body movements, decision-making, reward and addiction. The basal ganglia contains a high density of mu-opioid receptors, which may contribute to the increased concentrations of fentanyl in this region. The medulla, pons, and cerebellum, which had the lowest concentrations of fentanyl, contain lower densities of mu-opioid receptors. These results will help pathologists to determine brain collection sites when fentanyl use is suspected. Future work will include evaluation of fentanyl brain:blood ratios as fentanyl is known to exhibit postmortem redistribution.

P-007

Double Designers: Detection of Bromazolam and Metonitazene in Postmortem Casework

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Abstract

Introduction: Novel psychoactive substances (NPS) by their nature are a constant challenge for toxicology laboratories. Not only are the structures of these substances constantly changing, but their popularity amongst the public are unpredictable as well. There is no exception for the Los Angeles County Department of Medical Examiner (LACDME). Novel synthetic opioids (NSO) and novel benzodiazepines are amongst the most encountered NPS at the LACDME, sometimes together in a combination referred to colloquially as "benzo-dope". This presentation discusses a case in which an NSO and a novel benzodiazepine were the only substances detected and were solely attributed to the manner and cause of death. These substances were metonitazene and bromazolam. Metonitazene is a relatively new non-fentanyl NSO that has emerged in the illicit drug market and has been reported to have potency 100 times that of morphine and a similar or slightly higher potency than fentanyl. Bromazolam is the brominated analogue of alprazolam and has been linked to adverse effects involving hospitalization and death. This presentation will go over the process that it took to come to these findings, as it was atypical of the standard process for toxicology testing at the LACDME. Constraints in available screening and confirmation testing made detection difficult and ultimately the case heavily involved another section of our department, drug chemistry.

Objectives: The goal of this case review is to highlight the collaborative efforts between toxicology laboratories as well as the utilization of drug chemistry analysis in identifying NPS in biological specimens when routine analytes of interest are not detected in initial testing. Additionally, a portion of the presentation will be to discuss the techniques that may be available in the future at the LACDME that will hopefully enable the toxicology laboratory to detect NPS more easily.

Methods: The standard process for toxicology testing at the LACDME involves Enzyme-Linked Immunosorbent Assay (ELISA) for screening, and in this case benzodiazepine confirmation by Liquid Chromatography Tandem Mass Spectrometry (LC/MS/MS). The presentation also goes over the LACDME procedure for performing drug chemistry on medical evidence collected at the scene of death investigation.

Results: Jugular and femoral blood samples were submitted to an outside agency for quantitative toxicology testing. Metonitazene was detected in the jugular and femoral blood at 1.6 ng/mL and 4.4 ng/mL, respectively. Bromazolam was detected in the femoral blood at 93 ng/mL. No other drugs were detected, and the manner and cause of death were determined as accidental due to effects of bromazolam and metonitazene.

Discussion: The ever-growing landscape of NPS provides a real challenge for toxicology laboratories. To combat these challenges, it is essential for agencies to provide their laboratories with the means to keep their screening and confirmation techniques as up to date as possible. It is also vital for agencies to conduct a thorough on-scene investigation and document the presence of unknown substances so they may also be tested. The presented case highlights the importance of toxicology laboratories to be vigilant, adapt and strive to improve as NPS can easily go undetected in routine casework.

P-008

Increasing Concentrations of Fentanyl in Driving Cases in Nashville TN, a Retrospective Study from 2015-2024

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Abstract

Introduction: Fentanyl, a potent synthetic opioid, has become a major contributor to the opioid crisis and a threat to public safety. Fentanyl's use has surged over the past decade and concerns have grown regarding its impact on road safety due to the increase detection in impaired driving cases. The high numbers of opioid related deaths caused the US Government to declare a national public health emergency in 2017.

Objectives: This study retrospectively examined trends in fentanyl-related driving cases in Nashville and Davidson Counties from 2015 to 2024. The study focused on both the frequency of cases and the concentration levels detected in drivers.

Methods: Data from confirmed fentanyl-positive driving cases were collected and reviewed, including the number of cases per year and the measured fentanyl concentrations in blood samples.

- 2015-2021 samples were screened via LC/MS/MS-QTRAP (LOQ 1ng/mL) and confirmed via LC/MS/MS (LOQ 1 ng/mL).
- 2021-2024 samples were sent to NMS for testing.

Statistical analysis was performed to assess trends over time using Excel.

Results: Over a nine-year period, 8,933 cases were all analyzed for alcohol and drugs, 914 (10%) were confirmed positive for fentanyl. From 2015 to 2017 the percentage of fentanyl positive cases was less than 3%. After 2017, those numbers continued to increase, with 2021 being the highest percentage of fentanyl positive cases, at 22% of cases. Additionally, the concentration of fentanyl detected in drivers has shown an upward trend. From 2015-2021, concentrations slowly increased ranging from 0.41 ng/mL – 82 ng/mL, average 6.8 ng/mL and median of 5.6 ng/mL. However, from 2022-2024 fentanyl concentrations rose sharply ranging from 0.26 ng/mL – 380 ng/mL, with average concentrations over 15 ng/mL and median of 8.1 ng/mL. The total percentage of positive fentanyl cases has slowly begun to decline, which is in line with what other laboratories across the U.S. are seeing, though levels have remained elevated through 2024. Polydrug use was common, with frequent co-detection of amphetamine, methamphetamine, and cocaine.

Case study #1:

Fentanyl: 220 ng/mL

Norfentanyl 190 ng/mL

Acetyl Fentanyl 0.34 ng/mL

Amphetamine: 150 ng/mL

Officers observed a 31-year-old male with the engine running. The suspect showed signs of impairment. This included bloodshot eyes, slurred speech, and dilated pupils. The suspect attempted to flee on foot and was apprehended. The vehicle was searched, and drugs were found. No SFSTs were performed and it is unknown whether naloxone was administered.

Case study #2:

Fentanyl 47 ng/mL

Amphetamine 34 ng/mL

Methamphetamine 640 ng/mL

Benzoylecgonine 64 ng/mL

Norfentanyl 15 ng/mL

A 48-year-old male with outstanding warrant was observed dropping off a known prostitute. A traffic stop was then initiated, the officer observed the suspect to be unsteady as they exited the vehicle, had constricted, glassy/watery eyes, and were talkative. Partial SFSTs were performed, for the WAT 5/8, stumbled during the turn and stepped off the line. For the OLS, officer noted that they did not keep the bottom foot flat on the ground and didn't look at the foot, however, no score was given. Suspect did admit to taking heroin and a pack of "heroin" was found.

Discussion: Variability in concentration levels were observed, with some cases exceeding thresholds associated with possible impairment or overdose risk. Findings indicate a significant increase in fentanyl-positive impaired driving cases from 2015 to 2024, aligning with the broader opioid crisis. Additionally, the average concentration of fentanyl in drivers has increased, suggesting a potential for an increase in tolerance and the availability of naloxone. The rising prevalence and increasing concentration of fentanyl in impaired drivers present critical challenges for traffic safety and law enforcement and important that laboratories and law enforcement work together.

P-009

Extraction of U.S. Department of Transportation Drug Testing Panel from Oral Fluid Using SPE Columns of Multiple Particle Sizes

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Abstract

Introduction: The U.S. Department of Transportation's (DOT) drug and alcohol testing program regulates substance testing for commercial transportation employees and employers. Previously only performed in urine, oral fluid was introduced as a viable matrix for DOT drug testing in May 2023. Oral fluid specimens must be tested for marijuana metabolites, cocaine metabolites, amphetamines, opioids, and phencyclidine. Mandatory cutoff concentrations for confirmatory tests range from 1 ng/mL to 20 ng/mL. Two years after approval of oral fluid for drug testing, no laboratories have been certified by the Department of Human and Health Services to conduct such testing for the DOT. This highlights the need for a comprehensive oral fluid method that can detect all analytes in the DOT drug testing panel at their obligatory cutoff concentrations.

Objectives: Develop analytical and extraction methods that can quantitate all analytes mandated by DOT drug testing. Analytes must be reliably detected at the confirmatory cutoff concentrations set forth in DOT Rule 49 CFR Part 40 §40.91. Use this method to compare solid phase extraction (SPE) sorbents of varying particle sizes to highlight the benefits of small, normal, and large particle columns.

Methods: A 1 mL aliquot of Quantisal™ oral fluid diluted in buffer (1:4) was further diluted with 2 mL 1% HCl before being added to a Clean Screen® DAU SPE column that had been previously conditioned with methanol and water. Columns were washed with 1% HCl, deionized water, and 25% acetonitrile. Columns were dried under pressure (~80 psi) using a positive pressure manifold before a dual elution with methanol followed by methanol: ammonium hydroxide (98:2 v/v) with an acetonitrile wash between elution steps. Samples were then evaporated under N_2 at 40°C and reconstituted in 150 µL of starting mobile phase conditions. The same extraction was performed by gravity using XtrackT® large particle DAU columns with pneumatic pressure only being applied during drying steps. Efficiency of small particle RSV DAU columns was also assessed using half the sample and solvent volumes.

Analysis was performed using a Shimadzu Nexera LC-30AD with MS-8050. Analytes were separated using a SelectraCore® DA column (100 x 2.1 mm, 2.7 µm) and SelectraCore® DA guard column (5 x 2.1 mm, 2.7 µm).

Results: Using normal particle size Clean Screen® DAU columns, the developed extraction exhibited recoveries of 68% to 105%. At DOT cutoff concentrations, bias and relative standard deviation (RSD) did not exceed 16%. Using large particle Xtrackt® columns, bias and RSD did not exceed 11% and recovery did not go below 70%. Performing the extraction using small particle RSV columns with half the sample and solvent volumes produced bias and RSD at the cutoff below 18%. Recovery was a minimum of 65% using RSV with analyte response being an average 10% lower than double the sample and solvent volumes when extracted using Clean Screen® DAU. Internal standard-normalized matrix effects ranged from -21% to 28% across the three sorbents.

Discussion: The developed methods can extract, detect, and quantitate all mandatory DOT drug-testing analytes from oral fluid samples. Large particle columns have lower surface area for analyte-sorbent interaction, but recovery was not negatively impacted using Xtract® columns. Extracting under gravity-flow also did not increase the variance between samples. These columns offer higher flow and lower back pressure making them a great option for laboratories testing viscous neat oral fluid or without access to positive pressure manifolds. Small particle SPE columns have higher surface area and were shown to achieve similar recoveries to the normal particle columns using half the sample and solvent volumes. RSV columns are a great option for labs looking to conserve sample or reduce solvent usage.

Conflict of Interest

2) Salary/Consultant

P-010

Bridging the Gap Between Biological Variability and Analytical Precision with Synthetic Matrices

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Abstract

Introduction: The development of synthetic matrices is essential to improving the accuracy and reliability of forensic toxicology testing. Traditional biological matrices can introduce variability that complicates method validation, quality assurance, and instrument calibration. Synthetic matrices offer a controlled and reproducible alternative that can better support these critical functions.

Objectives: This study aims to develop and evaluate synthetic blank saliva and urine matrices that closely replicate the chemical characteristics of biological fluids. This was achieved through designing the synthetic matrices to represent underlying pooled matrices so that background levels in the synthetic matrix would mimic what is seen in casework and therefore minimise bias introduced into interpretations. The goal of this is to enhance the fidelity of toxicological testing environments and reduce the influence of matrix variability on analytical outcomes.

Methods: Recent advancements in synthetic matrix formulation were explored, focusing on mimicking the analyte profiles and matrix effects typically observed in toxicological casework. Key additives, such as creatinine, were incorporated to better align the chemical composition of synthetic matrices with that of authentic biological fluids. Candidate formulations were assessed for their suitability in method validation and calibration processes.

Results: The inclusion of physiologically relevant compounds, such as creatinine in the synthetic urine, improved the chemical resemblance of synthetic matrices to biological fluids. These enhanced formulations provided consistent, controlled testing backgrounds, reducing variability and increasing reproducibility in analytical workflows. Preliminary evaluations indicate that the matrices perform well in simulating real-case conditions for both qualitative and quantitative assessments.

Discussion: By reducing reliance on authentic biological samples, synthetic matrices offer a practical and scalable solution for forensic laboratories. Their use improves standardization, facilitates more robust method development, and supports long-term quality control efforts. Continued refinement of their composition could further enhance their utility across various toxicological applications.

P-011

Development of an Analytical Method for Targeted Screening of Eutylone, N,N-Dimethylpentylone and Pentylone in Dried Blood Spots using Liquid Chromatography-Triple Quadrupole Mass Spectrometry (LC-MS/MS)

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Abstract

Introduction: Microsampling techniques have recently gained more applications in the forensic toxicology landscape after long-time applications in clinical settings. Dried blood spots (DBS) provide the ability for fast and easy sampling with minimal transportation and storage requirements. DBS can be valuable in the toxicological analysis of three synthetic cathinones, eutylone, *N,N*-dimethylpentylone (DMP) and pentylone, which have been frequently reported in forensic casework.

Objectives: The goal of this study was to develop a method for screening eutylone, DMP and pentylone in DBS.

Methods: Bovine whole blood was spotted onto pre-cut Whatman® protein saver cards and dried for 2h, protected from light. DBS were further extracted and reconstituted in 100 µL of mobile phase at initial conditions. The type of incubation (agitation or sonication), extraction solvent (methanol, 1% HCl in methanol, or methanol with 0.1M borate buffer pH 9) and time (10, 20, or 30 min) were optimized. Analyses were performed using an Agilent 1290 Infinity II liquid chromatograph coupled to an Agilent 6470 triple quadrupole mass spectrometer, using positive ESI and multiple reaction monitoring mode. Pentylone-d_3 was used as the internal standard (ISTD). Chromatographic separation was performed using an Agilent Poroshell 120 EC-C18 column (2.1 x 100 mm, 2.7 µm) and a gradient elution adapted from literature (1). LC gradient and MS parameters were optimized for all compounds. A fit-for purpose method validation was performed based on the ANSI/ASB Standard 036 (2).

Results: Method optimization indicated no statistically significant differences in analyte's response when testing different types of incubation, extraction times, and extraction solvents. Extraction via sonication with methanol for 20 min was selected based on better reproducibility and sample preparation convenience. Matrix effects (ME) were assessed (3) over two days, using six blood sources, in a single replicate (day 1) and duplicate (day 2). Average ME ranged from 105% to 109% for the low quality control and from 100% to 102% for the high quality control. Coefficients of variation (CV) for ME on day 2 and between both days were 7.6% or lower. Recovery was assessed over two days, using six blood sources and ranged from 62.8% to 114%. Recoveries were considered acceptable (> 60%), despite CV greater than 20%. The CV for peak areas of pre-spiked samples and average recoveries across the two runs were in the range of 5.20 - 25.7% and 18 - 22%, respectively. A minor interferent peak for DMP from pentylone-d_3 was observed. An administratively set cutoff of 10 ng/mL was assessed in triplicate in three blood sources. To ensure the interferent peak from pentylone-d_3 for DMP did not impact the limit of detection, an additional analysis without pentylone-d_3 was performed, and all analytes met the positive identification criteria over all four days. Alternatively, DMP-d_6 could be explored as the

ISTD. No carryover was observed after injecting a sample at 1,000 ng/mL. No interferences from 10 different blood sources nor common drugs (cannabinoids, MDA, MDMA, methamphetamine, PCP, phenylpropanolamine, benzoylecgonine, cocaethylene, cocaine, methadone, diazepam, nordiazepam, oxazepam and temazepam) were observed. Analytes remained stable post-processing for 72h in the autosampler at room temperature.

Discussion: The analysis of synthetic cathinones in DBS offers several advantages such as minimal sample preparation and reduced solvent use and waste. This method exhibited acceptable sensitivity and selectivity for screening the target compounds to be applied to the analysis of authentic specimens.

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P-012

Comparison of Thermally Modified Polar C18 and Superficially Porous Biphenyl LC Columns for Confirmation of Prescribed Drugs and Metabolites by LC-MS/MS

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Abstract

Introduction: Correct identification and quantitation of prescribed drugs and metabolites is important in toxicology laboratories for medicolegal casework. Here, two columns: a fully porous, thermally modified C18 LC column with a polar modified surface was compared to a superficially porous "core shell" biphenyl LC column for LC-MS/MS analysis of three panels of prescribed drugs and metabolites: 22 antidepressants, 14 antipsychotics and 11 anticonvulsants, for a total of 47 target compounds. These three drug classes encompass analytes that are polar to moderately hydrophobic, acids, bases, zwitterions, and isobaric compounds. This range of properties can make development of a fast, yet robust LC method for a multi-analyte panel challenging. This comparison determined the better column and conditions for fast LC separation for each drug class, while achieving baseline resolution of isobaric compounds.

Objectives: To determine the better LC column and conditions for three drug classes encompassing 47 prescribed drugs and metabolites for LC-MS/MS confirmation.

Methods: Two 50 x 3.0 mm columns: a fully porous, thermally modified Luna Omega 3.0 mm Polar C18, and a core-shell Kinetex 2.6 mm Biphenyl column were compared. The Biphenyl column used 0.1 % formic acid in water as mobile phase A, and the Luna Omega column used 2 mM ammonium acetate in water as mobile phase A. Methanol was used as mobile phase B for both columns. Gradient elution, delivered with an Agilent 1290 Infinity series LC system coupled to a SCIEX 6500 QTRAP mass spectrometer using ESI was employed for detection.

Results: The Biphenyl column was better for the separation of the antipsychotic panel, which included mostly basic and relatively hydrophobic compounds. Furthermore, the thermally modified fully porous column failed to differentiate between the isobaric antipsychotic analogs Norclozapine and Olanzapine. Conversely, the analytes in the anticonvulsant panel encompassed a wide range of polarities and chemical properties, including zwitterionic, weakly acidic, and neutral compounds. The better separation for these compounds was using the Luna Omega column. The superiority of this fully porous, thermally modified stationary phase for the anticonvulsants was demonstrated with baseline separation of the isobaric analytes Oxcarbazepine and Carbamazepine Epoxide. Both columns showed successful separation of the two isobaric pairs Venlafaxine and Amitriptyline, and Mirtazapine and N-desmethy-Doxepin in the antidepressant panel. However, higher MS signal response and wider separation of the isobaric analytes were observed with the Biphenyl column.

Discussion: The thermally modified fully porous Luna Omega particle enhanced the selectivity and retention of polar and neutral analytes, making it suitable for analysis of the more challenging anticonvulsant panel. The high efficiency Kinetex Biphenyl column demonstrated better separation of antipsychotics and antidepressant panels with good resolution of the critical isobaric pairs.

Conflict of Interest

The authors are paid employees of Phenomenex and its products are used as examples.

P-013

Hydrolysis of Drug Glucuronides by β-Glucuronidase is Dependent on Both pH and Type of Sugar Linkage to Drug Aglycones

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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 by hydrolysis with a β-glucuronidase (β-GUS) and/or sulfatase. The activity of the β-glucuronidases will be affected both by the pH of the solution and the aglycone moiety of the drug glucuronide. Furthermore, for a single β-GUS, different drug glucuronides may display different pH optimums which is a consequence of aglycone functional groups and glucuronide linkage.

Objectives: Compare the activities of commercially available β-glucuronidase enzymes on O-linked and N-linked drug glucuronides in different pH buffers.

Methods: Deuterated and reference standards for morphine, oxymorphone, codeine, O-desmethyltramadol, O-desmethylvenlafaxine, amitriptyline, and nortriptyline were purchased from Cerilliant. All other reagents were purchased from MilliporeSigma or Fisher Scientific. Four β-glucuronidases were expressed and purified at IMCS: modified *Escherichia coli* β-GUS (E1F), *Aspergillus* chimera β-GUS (R3), *Eubacterium eligens* β-GUS (EeGUS), and *Brachyspira pilosicoli* (B. pi.) β-GUS variant (BpiGUS). A triple buffer system of Tris, Acetate, and MES was used from pH 4-9. Calibration curves include parent and glucuronide standards. Reactions were stopped by transferring sample into methanol (40%). 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: The pH optimum was dependent on both the enzyme and the linkage (O- or N-) of the sugar to the drug aglycone. For O-linked drug glucuronides, E1F and BpiGUS favored neutral to basic pH while β-GUS Variant R3 and EeGUS had preference in acidic buffers. However, when N-linked drug glucuronides were hydrolyzed by the same enzymes, the pH optima of all enzymes shifted towards a more basic pH.

Discussion: The β-GUS enzymes characterized here display variable pH optimums on drug glucuronides. Additionally, the pH optimum changes per enzyme based on whether the drug glucuronide is O-linked or N-linked, and the O-linked drug glucuronides are generally hydrolyzed at lower pH than N-linked drug glucuronides relative to each enzyme tested. The change in pH optimum based on sugar linkage may be related to the pKa of the amine in the N-linked drug glucuronides. When analyzing and comparing different β-GUS enzymes, the pH of the reaction buffer and sugar linkage of the target substrate(s) should be acknowledged in order to achieve optimal hydrolysis.

Conflict of Interest

I am an employee of Integrated Micro-Chromatography Systems and receive a salary.

P-014

Psilacetin's Stability Challenge: Understanding and Enhancing Its Detection in Blood

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Abstract

Introduction: Psilacetin (4-acetoxy-DMT), a synthetic prodrug of psilocin, is gaining popularity as a therapeutic and recreational psychedelic. However, its instability in biological matrices, primarily due to hydrolysis into psilocin, poses significant analytical challenges in forensic toxicology. Understanding how to preserve psilacetin in blood is essential for accurate detection during toxicological evaluations. This will allow differentiation of psilacetin exposure from psilocybin and other novel tryptamines, such as 4-propionoxy-N,N-dimethyltryptamine (4-PrO-DMT), which are also psilocin prodrugs. While antioxidants like ascorbic acid (AA) are known to offer some protection against oxidative degradation, the optimal concentration and compatible preservatives remain unclear.

Objectives: The goal is to determine the optimal concentration of the antioxidant AA and its combination with various preservatives that ensure the best stability of psilacetin in whole blood.

Methods: Using equal volumes of sheep plasma and AA, various concentrations of AA (25, 50, 125, 200, 250, 500, 1000 mM) were tested to identify the optimal stabilization of psilacetin at 100 ng/mL. Subsequently, 250 mM AA was selected for blood preservation studies. The matrices included human whole blood preserved with sodium heparin, sodium citrate, dipotassium ethylenediaminetetraacetate (K_2EDTA), and sodium fluoride/potassium oxalate (NaF/KOx), as well as bovine and sheep blood treated with sodium citrate and Alsever's solution.

Pooled samples of plasma and whole blood were prepared at 100 ng/mL (500 µL matrix + 500 µL AA + 25 µL Psilacetin at 2 µg/mL) in 1.5-mL Eppendorf tubes and incubated for 15, 30, and 60 minutes at room temperature. Three 100 µL aliquots (containing 50 µL plasma/blood + 5µL of the corresponding AA solution) were fortified with the internal standards (psilocin-d10 and psilocybin-d4 at 25ng/mL). The samples underwent protein precipitation with 250 µL of cold acetonitrile, followed by centrifugation, evaporation via TurboVap™ at 50°C, and reconstitution in 100 µL of mobile phase A (0.1% formic acid in water in 10 mM ammonium formate) and mobile phase B (0.1% formic acid in acetonitrile), in a 95:5 v/v ratio. Chromatographic separation was performed using a reversed-phase C18 column under gradient elution (0.3 mL/min, run time 10 minutes). From 1 to 7 minutes, B gradually increased to 50% composition, which was then rapidly increased to 90% composition at 7.01 minutes and maintained until 8 minutes, when it was decreased back to 5%. Detection was accomplished using a Shimadzu LCMS-8050 triple quadrupole mass spectrometer in positive electrospray mode, with monitoring of two MRM (multiple reaction monitoring) transitions per compound. The analytical method was validated following the guidelines outlined in ANSI/ASB Standard 036, Standard Practices for Method Validation in Forensic Toxicology.

Results: Preliminary studies of psilacetin in plasma and blood samples from various species (sheep, cow, human) and using different preservatives (citrate, Alsever's solution, and NaF/KOx) without AA demonstrated a complete and instantaneous (less than 5 minutes) hydrolysis of psilacetin to psilocin. Initial testing in plasma indicated that psilacetin stability improved with

increasing AA concentration; however, stabilization plateaued (based on psilacetin and psilocin concentrations) beyond 250 mM, with minimal added benefit at 500 mM and 1 M. Therefore, 250 mM was selected. Utilizing 250 mM AA, bovine and sheep blood preserved with sodium citrate and Alsever's solution exhibited high psilacetin stability across all time points, with minimal psilocin formation (≤1.7 ng/mL). In human blood, sodium heparin, sodium citrate, and K_2EDTA blood samples demonstrated rapid psilacetin hydrolysis, with psilacetin dropping to half of its initial concentration at 15 minutes. By 60 minutes, psilacetin decreased to 8–25 ng/mL, while psilocin rose to 44 ng/mL, indicating significant degradation of psilacetin. Interestingly, in the case of NaF/KOx blood, psilacetin remained stable, with psilocin concentrations at ≤2.2 ng/mL by 60 minutes at room temperature.

Discussion: This research confirms that both the concentration of AA and the choice of blood preservative significantly impact the stability of psilacetin. Equal volumes of 250 mM AA and bovine and sheep blood with citrate or Alsever's solution or NaF/KOx-preserved human blood demonstrated the highest psilacetin stability and the lowest levels of psilocin formation. Human blood samples preserved with sodium heparin, sodium citrate, and K_2EDTA showed rapid degradation, indicating that these preservatives are inadequate for maintaining analyte integrity.

P-015

Evaluation of the Neogen® Gabapentin Forensic ELISA for Human Matrices

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Abstract

Introduction: Gabapentin usage in the US has increased since its FDA approval in 1993. It has seen a rise in recreational use and an increase in prescriptions. [1, 2, 3] An Enzyme-Linked Immunosorbent Assay (ELISA) screening method that allows for direct monitoring of gabapentin in human matrices would be beneficial for forensic testing applications.

Objectives: This study reports the performance of a specific and sensitive ELISA for the detection of gabapentin in human oral fluid, whole blood, serum, and urine samples.

Methods: The Neogen Gabapentin Forensic ELISA was tested for sensitivity, reproducibility, and cross-reactivity in sample dilution buffer and matrix interference in negative populations of diluted human oral fluid, whole blood, serum, and urine. Matrices were diluted with corresponding buffer (oral fluid samples were diluted 1:4 with Oral Fluid Buffer and blood, serum and urine samples were diluted 1:20 with EIA Buffer) and tested according to product insert. Screening cutoff concentrations of 50 ng/mL (oral fluid) and 1,000 ng/mL (blood and urine) were used.

Results: The limit of detection of gabapentin in sample dilution buffer was 1.7 ng/mL. Human oral fluid (n=80), whole blood (n=74), serum (n=72), and urine (n=80) sample populations, presumed to contain no drug, were tested for assay interference and no false positives were observed at a 50 ng/mL (neat) screening cutoff in oral fluid and a 1,000 ng/mL (neat) screening cutoff for whole blood, serum, and urine. Percent cross-reactivity was calculated relative to gabapentin with pregabalin methyl ester, (-)-levamisole, pyrantel, pregabalin, and R(-)-methamphetamine having the highest relative cross-reactivity at 9.4%, 4.5%, 1.9%, 1.1%, and 0.1%, respectively. All other structurally related drugs tested had a cross-reactivity below 0.1%. The assay did not show cross-reactivity (< 0.1%) with a comprehensive selection of drugs including drugs of abuse, prescription and over the counter drugs. The reproducibility of the dose response curve was evaluated and found to be 10% or less at all concentrations tested. The concentration of gabapentin needed to inhibit the response by 50% (IC50) was 11 ng/mL.

Conclusions: The Neogen Gabapentin ELISA described is robust and sensitive for the testing of human oral fluid, whole blood, serum, and urine in a forensic setting. It allows for screening and direct monitoring of gabapentin in multiple matrices and concentrations analyzed by toxicology testing laboratories.

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Conflict of Interest

2. Salaried Employee. 3. Stock in company

P-016

Comparative Evaluation of Clean Screen® BNZ and Alternative Methods for the Extraction of Prescription and Designer Benzodiazepines

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Abstract

Introduction: Benzodiazepines are characterized by their chemical structure, which consists of a benzene ring fused to a seven-member diazepine ring. These drugs can be used to treat anxiety, insomnia, muscle spasms, and seizures. Unfortunately, they can also be misused or abused. This has led to the emergence of designer benzodiazepines (DBZD), which are novel psychoactive substances (NPS) illicitly manufactured and sold to evade legal restrictions. DBZDs are compounds structurally and pharmacologically similar to traditional benzodiazepines. Laboratories are faced with the challenge of continually updating and revalidating methods for the detection of these changing compounds. Methods for the simultaneous extraction and detection of prescription and designer benzodiazepines can help streamline workflow.

Objectives: The objective was to evaluate three extraction methods for traditional and designer benzodiazepines using Clean Screen BNZ® SPE columns, QuEChERS, and Refine™ ultra-filtration columns.

Methods: This poster outlines three extraction methods for a panel of eighteen prescription and designer benzodiazepines. Spiked blood and urine samples were extracted using UCT's Clean Screen BNZ® SPE column. Clean Screen® BNZ contains a proprietary sorbent that was specifically engineered for the retention of benzodiazepines and their 7-amino metabolites.

Spiked blood and urine samples were prepared using a QuEChERS-based technique. 15mL centrifuge tubes containing 400mg MgSO4 and 100mg sodium acetate were used to help partition the benzodiazepines into the organic acetonitrile layer from the aqueous matrix.

Spiked urine samples were prepared using the Refine™ ultra filtration SPE columns. Special formulation of the column allows for in-column enzyme hydrolysis. Hydrolysis recovery of known glucuronidated metabolites was assessed.

All samples were analyzed using a Shimadzu LC-MS/MS equipped with a 10cm superficially porous HPLC column, SelectraCore® DA (biphenyl). Mobile phase A composition was 5mM ammonium formate with 0.1% formic acid in water. Mobile phase B composition was 5mM ammonium formate with 0.1% formic acid in methanol. The extraction efficiencies, matrix effects, and relative standard deviations of each sample preparation method were compared. All spiked samples were prepared at 30 ng/mL and 300 ng/mL in replicates of five to evaluate extraction methods.

Results: Using the Clean Screen® BNZ extraction method, analyte recovery for blood and urine samples ranged from 58% to 103%. Matrix effect ranged from -30% to 17%, and relative standard deviation ranged from 0% to 17%.

The QuEChERS-based method resulted in analyte recovery for blood and urine samples ranging from 69% to 130%. Matrix effect ranged from -19% to 28%, and relative standard deviation ranged from 1% to 13%.

Using the Refine™ ultra-filtration columns for urine samples resulted in analyte recovery ranging from 80% to 94%. Matrix effect ranged from -21% to 15%, and relative standard deviation ranged from 1% to 11%. In-column hydrolysis recovery for lorazepam and oxazepam, two benzodiazepines known to undergo phase II metabolism, ranged from 76% to 108%.

Discussion: Each extraction method produces acceptable recoveries, matrix effects, and relative standard deviations. The traditional SPE method is highly effective for both matrices and theoretically compatible with GC and LC analysis. It also allows for high-throughput processing, as manifolds can accommodate multiple columns for the simultaneous extraction of large batches. The QuEChERS method is also suitable for both matrices and offers compatibility with GC and LC analysis. Other advantages of this method include reduced solvent usage and faster extraction time. However, this technique poses challenges when extracting batches with a large number of samples. Lastly, the Refine™ method is only compatible with LC analysis of urine samples. However, the in-column hydrolysis feature helps simplify the workflow and reduce lab consumables.

Conflict of Interest

Salary

P-017

Deaths Involving the Inhalation of Chloroethane

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Abstract

Introduction: The abuse of inhalants, including solvents, aerosols, gases, and nitrites, can cause a variety of effects, including euphoria, dizziness, and headache, but can also lead to death from neurotoxicity. Chloroethane, or ethyl chloride, is a volatile hydrocarbon that can be found as a topical anesthetic, usually in a spray form, and in cleaning products.

Objectives: To share two postmortem cases in which a unique volatile was identified.

Methods: Two replicate samples from the same blood tube were prepared using a Hamilton ML600 dual syringe diluter. For each replicate, 250µL of blood and 1.5mL of ~0.05% n-propanol internal standard was dispensed into a glass headspace vial and capped. After an ethanol and acetone calibration curve was completed and evaluated, the prepared samples were analyzed on an Agilent 8890 GC-FID, using an Agilent 7697A Headspace Sampler, on dual columns (Restek RTX-BAC PLUS 1 and RTX-BAC PLUS 2). Data review was completed in Agilent OpenLab CDS Chemstation. An aliquot of blood from each case was sent to a private lab for confirmation.

Results:

Case #1: 2 gray-stoppered tubes containing iliac blood and 1 red-stoppered tube containing urine collected at autopsy

- > 25 mcg/mL chloroethane
- 0.011 g/dL EtOH
- Cannabinoids indicated in urine only (cannabinoids are not confirmed in postmortem urine toxicology analysis)

Case #2: 2 gray-stoppered tubes containing iliac blood collected at autopsy

- > 25 mcg/mL chloroethane
- 91 ng/mL methamphetamine
- 62 ng/mL amphetamine
- 0.45 mg/L gabapentin

During the death investigations, MAXIMUM IMPACT Cleaning Solvent, an aerosol spray with chloroethane used to clean audio, visual, and data-related electronic equipment, was found at both scenes.

Discussion: The 2022 death of Chauncy Glover, a news anchor from Los Angeles, and the 2024 death of Darius Campbell Danesh, a British singer and actor, were both determined to be accidental with chloroethane inhalation being a contributing factor in their deaths. Two postmortem cases are presented here, in which chloroethane was found in the blood samples collected at autopsy, which likely contributed to the cause of death in both individuals.

P-018

Sensitive Quantitation of Nine Nitazene Analogs and Brorphine in Dried Blood Spots

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Abstract

Introduction: The introduction of highly potent novel synthetic opioids (NSO) to the recreational drug market has been contributing to the major rise in the number of accidental drug overdoses. In recent years, a class of synthetic opioids originally developed in the 1950s called nitazenes has emerged in the illicit drug supply. Brorphine is predominately used with, or as an alternative, to the nitazene, isotonitazene. Further, it is commonly detected with other synthetic opioids. In this study a comprehensive workflow for the sensitive quantitation of 9 nitazene analogs and brorphine in dried blood spots (DBS) was developed. DBS provide several advantages as compared to conventional blood sampling methods. For example, DBS sampling is less invasive than intravenous blood sampling and the logistics of storage, handling and transport of DBS is much simpler.

Objectives: To develop a workflow for the analysis of 9 nitazene analogs and brorphine in DBS using LC-MS/MS with only 10 µL of blood.

Methods: A panel of 10 analytes, including 9 nitazene analogs and brorphine, as well as fentanyl-D5, used as internal standard, were spiked in human whole blood samples at concentrations ranging from 1 to 50 ng/mL. 10 µL spots were generated on DBS cards and extracted using 500 µl of 3:1 (v/v) methanol/acetonitrile with fentanyl-D5 as the internal standard. Liquid chromatography was performed using a SCIEX ExionLC AC system and mass spectrometry analysis was performed on a SCIEX 7500 system where MS and MS/MS data were acquired using positive electrospray ionization. A single acquisition method consisting of 3 MRMs per nitazene analyte and 2 MRMs for brorphine was created and used for analysis of the samples. The method validation followed a previously published protocol and performance parameters included the calibration curve, intra- and inter-day accuracy and precision at all calibration levels, limit of detection (LOD) and quantitation (LOQ) as well as method recovery, matrix effects and analyte stability.

Results: A series of 6 spiked calibrator blood samples ranging from 1 to 50 ng/mL were extracted and injected in triplicate. The lower limits of detection (LLOD) for the 10 analytes included in the panel ranged from 0.3 to 0.5 ng/mL. These values are comparable to reported studies using larger whole blood sample volumes. Excellent linearity was observed across the concentration ranges analyzed with R^2 values greater than 0.99 for all the analytes using $1/x^2$ weighting. Linearity was verified using Mandel and lack-of-fit tests. The average accuracy was 10% (bias) and precision was within 15%CV for all compounds. The matrix effect was found to be between 85 and 115%, which resulted in ion suppression values corrected by internal standard between -15 and 15%. Despite being low (between 15 and 20%), the recovery values were reproducible across the calibration range.

Discussion: A fast and simple extraction procedure, combined with a robust acquisition method enabled the sensitive and accurate quantitation of the 9 nitazenes and brorphine. Using the high-end LC-MS/MS system detection limits ranging from 0.3 to 0.5 ng/mL were achieved, starting from a sample volume of only 10 µL of blood.

P-019

Tissue Distribution of THC and Its Metabolites in Rats Following Exposure to a Vaped THC

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Abstract

Introduction: Delta-9-Tetrahydrocannabinol (THC), the principal psychoactive constituent of cannabis, is increasingly consumed through vaping. This mode of administration is favored for its rapid onset of effects. THC is rapidly absorbed through the pulmonary system, reaching peak plasma concentrations within minutes. THC undergoes extensive hepatic metabolism, primarily via cytochrome P450 enzymes (CYP2C9 and CYP3A4), resulting in metabolites such as 11-hydroxy-THC (11-OH-THC) and 11-nor-9-carboxy-THC (THC-COOH). These metabolites are detectable in various biological matrices and are widely used as biomarkers of THC exposure in forensic and clinical toxicology. The distribution of THC and its major metabolites, 11-OH-THC and THC-COOH, in rat serum and six tissues (brain, heart, kidney, liver, lung, and spleen) was investigated following daily exposure to vapor from 200 mg/mL THC in 100% propylene glycol. Samples were collected approximately 5 and 30 minutes after the final THC exposure to characterize distribution of THC and its metabolites.

Objectives: To investigate the distribution of THC and its primary metabolites in rats following inhalation exposure to vaporized THC.

Methods: Two female Sprague Dawley rats were exposed daily over a period of 20 days to vaporized tetrahydrocannabinol (THC) in 100% propylene glycol using a vapor inhalation system (La Jolla Alcohol Research, Inc.). Each exposure consisted of 10 puffs (6 seconds each) of 200 mg/mL THC evenly distributed over a 30-minute session, delivered via e-vape-type tanks. Serum and tissue samples (brain, heart, kidney, liver, lung, and spleen) were collected on day 20 and stored at −80°C until analysis.

Tissue samples were diluted in deionized water (1:4, m/v) and homogenized using an Omni Bead Ruptor (Omni International Inc., Kennesaw, GA). Seven-point calibration curves were prepared for THC (1–250 ng/mL or ng/g), 11-hydroxy-THC (11-OH-THC), and 11-nor-9-carboxy-THC (THC-COOH) (5–1250 ng/mL or ng/g) in serum or brain homogenate. Quality control (QC) samples were prepared at 3, 30, and 75 ng/mL or ng/g, along with negative and blank controls in serum and tissue homogenates. Samples were prepared by adding an internal standard containing 10 ng of THC-d_3 and 50 ng each of 11-OH-THC-d_3 and THC-COOH-d_3 to 100 µL of serum or 400 µL of tissue homogenate. Samples were extracted with cold acetonitrile, evaporated to dryness, and reconstituted in 100 µL of mobile phase.

Analyses were performed using a Sciex ExionLC 2.0+ liquid chromatography system coupled to a Sciex 6500 QTRAP mass spectrometer. Chromatographic separation was on a Zorbax Eclipse XDB-C18 column (4.6 × 75 mm, 3.5 µm) with 0.1 mM ammonium formate/methanol (10:90, v/v) at a flow rate of 1 mL/min. The system operated in multiple reaction monitoring (MRM) mode. Precision and accuracy for all calibrators, controls and analytes yielded coefficients of variation (CV) < 20%.

Results:

Post-Vape Collection (~5 min)					**Post-Vape Collection (~30 min)**			
Sample	THC	11-OH-THC	THC-COOH		Sample	THC	11-OH-THC	THC-COOH
Serum	212	10.7	8.2		Serum	121	7.6	27.7
Brain	52.9	28.4	2.7		Brain	42.1	26.4	6.4
Liver	60.2	288	217		Liver	49.1	102	192
Lung	1860	90.4	36.2		Lung	322	24.2	20.0
Heart	19.8	6.5	7.6		Heart	11.1	7.2	10.9
Spleen	29.4	7.5	5.2		Spleen	9.3	5.9	8.5
Kidney	208	55.8	28.6		Kidney	75.9	70.0	50.9
Results reported in ng/mL or ng/g								

Discussion: The results demonstrated elevated concentrations of THC in samples ~5 minutes post-exposure samples compared to ~30 minutes. Lung tissue exhibited markedly higher concentrations of THC than other tissues. 11-hydroxy-THC (11-OH-THC) and 11-nor-9-carboxy-THC (THC-COOH), were detected across all tissues, with the highest concentrations observed in the liver and kidneys.

Funding

National Institutes of Health (P30DA033934), National Institute of Justice (15PNIJ-23-GG-01421-COAP) and the Johns Hopkins Children's Center Research, Education, and Patient Care Innovation Grant

P-020

Strategies for Mitigating the Effects of High Gabapentin Concentrations in Urine Specimens

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Abstract

Introduction: Gabapentin is an anti-convulsant drug prescribed for the treatment of neuropathic pain and seizures, as well as for many off label uses. Gabapentin is prescribed in high doses relative to other therapeutic drugs and is eliminated in urine predominantly in its unchanged form, which often results in extremely high concentrations of this compound occurring in patient urine samples. When analyzed by LC-MS/MS, high concentrations of gabapentin can have significant analytical implications, particularly for the compound amphetamine. Interference between gabapentin and amphetamine has been well documented and can result in signal suppression, poor peak shape, and shifting retention times. Other analytical challenges include saturation of the mass spectrometer and column overload. In this work, we explored several strategies to mitigate the effects of high gabapentin concentrations in urine samples.

Objectives: The objective of this study was to capture the analytical challenges presented by high concentrations of gabapentin in urine samples when analyzed by LC-MS/MS and investigate different strategies to mitigate them.

Methods: Using a Shimadzu LCMS-8045 triple quadrupole mass spectrometer in ESI+ mode, a method developed for the analysis of 60 drugs of abuse in urine was used to test two samples: one containing 0.1 µg/mL of both gabapentin and amphetamine, and one containing 250 µg/mL of gabapentin and 0.1 µg/mL of amphetamine. After data collection, the signal of amphetamine was compared in each sample to determine if interference from gabapentin was occurring. The method utilized a Raptor Biphenyl 50 x 2.1 mm, 2.7 µm column with a mobile phase A of water and mobile phase B of methanol, both modified with 0.1% formic acid. The flow rate was 0.6 mL/min, the column temperature was 45°C, and the injection volume was 5 µL. Gradient elution was employed, with a total runtime of 9 minutes. Following analysis, different strategies were tested to see if the interference between gabapentin and amphetamine could be resolved. These strategies included using alternate column lengths and diameters, decreasing injection volumes, adjusting the analyte dependent MS settings, and testing different mobile phase additives.

Results: Under the original method conditions tested, amphetamine showed a diminished signal and shifted retention time when a high concentration of gabapentin was present. The method was redeveloped using the strategies described. It was determined that the best approach for reducing interference was to fully chromatographically resolve gabapentin and amphetamine and ensure that gabapentin was the first compound to elute. This was done by switching the additive in mobile phase A from 0.1% formic acid to 10 mM ammonium formate, which affected the elution order of early eluting compounds. Resolution was further improved by switching from a 50 x 2.1 mm column to a 100 x 2.1 mm column. Detector saturation and column overload were improved by adjusting the analyte dependent MS settings for gabapentin and reducing the injection volume from 5 µL to 2 µL. Significant carryover was observed due to the high analyte concentrations and was eliminated by adding a small amount of 2-propanol to mobile phase B. The performance of the other analytes in the method was evaluated to ensure

that they were not negatively affected by the change in method parameters.

Discussion: Interference between gabapentin and amphetamine, chromatographic overload, and detector saturation were all observed when analyzed under the original method conditions. Altering the mobile phase composition, using an extended column length, adjusting the analyte dependent MS settings for gabapentin, and reducing the injection volume were all successful in mitigating these analytical challenges. The addition of 2-propanol to mobile phase B helped to reduce carryover by washing contaminants off the analytical column more efficiently. The redeveloped method can be used to effectively analyze 60 compounds in urine without interference between gabapentin and amphetamine.

Conflict of Interest

Employment

P-022

Quantification of Buprenorphine in Dried Blood Spots

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Abstract

Introduction: Buprenorphine is an important tool for treating opioid use disorder (OUD). Successful therapy relies on patient adherence, with >80% adherence associated with a reduced relapse risk. However, non-adherence is common, particularly in populations lacking routine access to standard clinical laboratory facilities, such as unhoused individuals. Objectively assessing patient adherence in these populations requires novel sample collection approaches designed to meet patients where they live. Dried blood spots (DBS) offer an alternative sample collection approach that can be applied within a mobile clinic setting, all without the need for standard clinical laboratory equipment associated with venipuncture blood collection.

Objectives: To assess the utility of DBS for measuring buprenorphine concentrations outside traditional clinical laboratory settings by defining the correlation between plasma and DBS buprenorphine concentrations.

Methods: Paired plasma and DBS samples were obtained via venipuncture from healthy adults participating in a pharmacokinetic (PK) study of oral buprenorphine (NCT 06086275). Buprenorphine was quantified in plasma and DBS samples using liquid chromatography-tandem mass spectrometry (LC-MS/MS). The plasma assay extracted a 50 µL aliquot by protein precipitation and achieved a lower limit of quantitation (LLOQ) of 0.1 ng/mL. The DBS assay utilized a 6 mm punch and an alkaline liquid-liquid extraction to achieve an LLOQ of 0.3 ng/mL. Data visualization and statistical analysis were performed in GraphPad Prism v10.3.1.

Results: A total of 175 paired plasma and DBS samples were collected from 9 participants. Plasma and DBS buprenorphine concentrations were quantifiable in 123 and 64 samples, respectively. Of the samples with a quantifiable plasma concentration (but a non-quantifiable DBS equivalent), 47 of 59 had concentrations <0.5 ng/mL. Linear regression analysis demonstrated that buprenorphine concentrations in DBS were 0.66 times that of plasma. Approximately 90% of the collected data was distributed evenly along the bias line determined from a Bland-Altman analysis, indicative of limited systematic bias within the assay of the two matrices. Further, the Bland-Altman analysis found 95% of the paired samples were within the 95% confidence interval around the mean concentration ratio between the two assays, supporting robust agreement between these two approaches.

Discussion: Innovation in blood sampling techniques provides institutions, such as mobile clinics, with an alternative to traditional venipuncture sampling. DBS is an approach that can be applied within such mobile clinics, which, from a therapeutic standpoint, provides these clinics with a more convenient way to track patient adherence. Despite the analysis of a lower blood volume (~20 µL whole blood in a 6 mm punch) relative to plasma, and the associated compromise in sensitivity, the DBS assay achieved an LLOQ lower than the therapeutic buprenorphine target concentration for managing OUD of >1 ng/mL. DBS concentrations are ~0.66 times that of plasma, suggesting negligible sequestration of buprenorphine into red blood cells. Further work

in understanding buprenorphine adherence in vulnerable populations, such as the unhoused, by a DBS assay, is still ongoing.

P-023

Δ8 vs. Δ9-THC: Detection, Quantification, and Forensic Implications in Human-Performance Toxicology

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Abstract

Introduction: Δ8-tetrahydrocannabinol (Δ8-THC) is a positional isomer of Δ9-tetrahydrocannabinol (Δ9-THC), differing in the location of the double bond within the alicyclic ring. The Agriculture Improvement Act of 2018 (2018 Farm Bill), which defines hemp as any part of the plant *Cannabis sativa L.* with a Δ9-THC concentration of not more than 0.3% of dry weight, distinguishes Δ9-THC and Δ8-THC. This federal "loophole" has led to an increase in products containing Δ8-THC, marketed as a "legal" high. Discrepancy between federal and state legality may have also led to an increased incidence of Δ8-THC and its metabolites appearing in forensic samples, with some samples having Δ8-THC as the presumed primary psychoactive agent instead of Δ9-THC. Some data suggests the effects of Δ8-THC are like Δ9-THC, but with decreased potency. Given the legalization of recreational cannabis use in some states, the rise in medicinal cannabis use, and changing attitudes towards cannabis, the use of cannabis and cannabis-derived compounds has been increasing, and their detection and differentiation are of importance in forensic casework.

Objectives: This project applies a validated quantitative method to evaluate Δ8-THC and its metabolites in blood samples, demonstrating its application to and impact on antemortem forensic casework.

Methods: The blood specimens were submitted to the University of Miami (UM) Forensic Toxicology Laboratory (UMFTL) as evidence in human-performance criminal investigations. All tested blood specimens were screened for cannabinoids using a targeted LC-MS/MS screening method. If the specimens were positive or presumptive positive for THC or its metabolites, they were reflexed for the blood cannabinoid quantitation and confirmation method. The laboratory implemented this method in October 2024. UM approved this study, IRB protocol 20040392.

Whole blood samples (1 mL) were fortified with an internal standard mixture, then allowed to equilibrate at room temperature for 30 minutes. Protein precipitation was carried out with 2 mL of cold acetonitrile, followed by centrifugation. The resulting supernatant was transferred to a clean tube, and 1.5 mL of deionized water was added to the sample before solid-phase extraction (SPE) using Cerex®THC columns. Following column conditioning and sample loading, the columns were washed and dried, then target cannabinoid compounds eluted. The eluents were evaporated to dryness under nitrogen and reconstituted in an appropriate solvent before derivatization with N,O-Bis(trimethylsilyl)trifluoroacetamide (BSTFA) containing 1% trimethylchlorosilane (TMS).

Quantitative analysis was conducted using gas chromatography-tandem mass spectrometry (GC-MS/MS). Samples were injected onto a GC column via splitless injection with helium as the carrier gas. A temperature ramp program was utilized for chromatographic separation. Detection was achieved using multiple reaction monitoring (MRM) for confirmation and quantitation, with 2 to 4 transitions monitored for each analyte using collision energies between 5 and 35 volts. The method was validated in accordance with ANSI/ASB Standard 036.

Results: Since implementation, Δ8-THC or its metabolites have been detected and reported in 21 cases out of 73 that were analyzed. To date in 2025, 43 out of 336 cases (12%) were tested for cannabinoids using this method, and 14 of those 43 (32%) had Δ8-THC or its metabolites. 13 of the cases (62%) were Drug Facilitated Crimes, while the remaining 8 (38%) were Driving Under the Influence cases. Seven out of the 21 cases (33%) were reported as cannabis positive only.

Table 1: Summary of blood concentration and assay conditions

Target	Number of Cases (n)	Linear range (ng/mL)	Limit of quantification (ng/mL)	Mean conc	Range
Δ9-THC	15	0.5-25	0.5	6.5	0.9-21
Δ8-THC	13	0.5-25	0.5	1.8	0.5-4.5
Δ9-THC-COOH	16	5 – 250	5	82	13-191
Δ8-THC-COOH	20	5- 250	5	39	6-428
11-OH- Δ8-THC	2	0.5-25	0.5	0.6	0.5-0.7
11-OH- Δ9-THC	14	0.5-25	0.5	4.1	0.5-23

Discussion: Cannabinoid use and its legalization represent a complex intersection of science, medicine, law, and public policy. With Δ8-THC and its metabolites now being identified in DUID and DFC cases, either alone or in greater or equal amounts to Δ9-THC and its metabolites, its detection and quantitation, along with its toxicological impact, has become increasingly important. This study was occasionally limited by low sample volume, high amounts of cannabis, and drug instability in matrix. The testing population was also limited as samples with a Blood Alcohol Content >0.150 g/100 mL are not drug tested as per protocol.

P-024

A Validated LC-MS/MS Method for Addressing Interferences of Norfentanyl by Synthetic Fentanyls in Urine

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Abstract

Introduction: The ongoing fentanyl crisis remains a significant public health concern in the United States, driven by the widespread abuse and misuse of fentanyl and its analogs. Fentanyl is primarily metabolized in the liver to norfentanyl. While unmetabolized fentanyl is rapidly eliminated, typically within four days, norfentanyl persists longer and is commonly used as a biomarker for fentanyl administration. In analytical toxicology, fentanyl and norfentanyl are typically quantified together via LC-MS/MS. We previously reported that three isomeric fentanyl analogs—ortho-, meta-, and para-methyl acetyl fentanyl—can interfere with fentanyl detection by LC-MS/MS. Furthermore, their potential metabolites or synthetic intermediates may interfere with norfentanyl identification and quantitation. Here, we present a validated LC-MS/MS method with alternative transitions that circumvents interference from norfentanyl isomers: ortho-, meta-, and para-methyl acetyl norfentanyls (designated as **A**, **B**, and **C**).

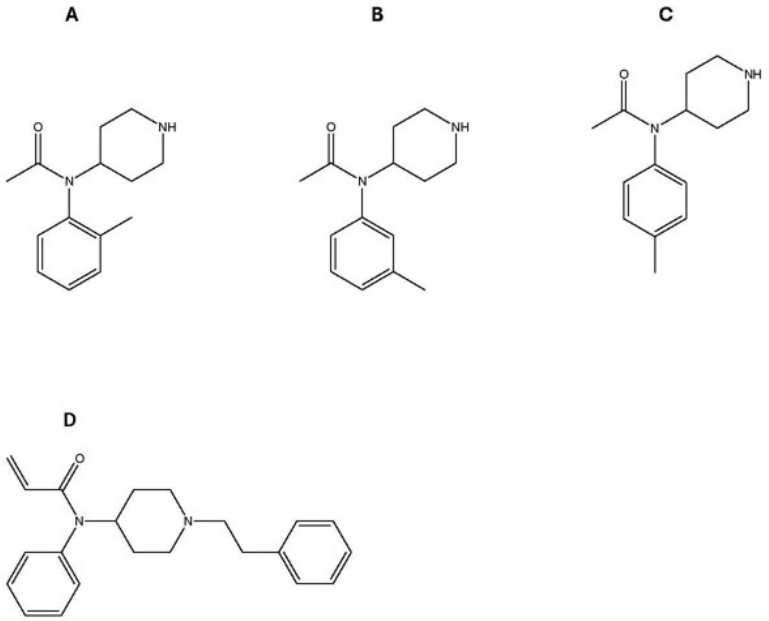
Objectives: To develop and validate an LC-MS/MS method for the quantification of fentanyl and norfentanyl in urine, compliant with Federal Workplace Drug Testing guidelines, and capable of resolving analytical interferences from structurally similar isomeric compounds.

Methods: Urine specimens (2.0 mL) were fortified with internal standards (fentanyl-d_5 and norfentanyl-d_5, each at 1.0 ng/mL) and subjected to solid-phase extraction using CEREX® Polycrom ClinII cartridges. Chromatographic separation was performed on an Agilent Poroshell Phenyl Hexyl column (4.6 µm × 50 mm) at a flow rate of 0.5 mL/min, with mobile phases consisting of 0.1% formic acid in water (A) and methanol (B). The gradient began at 30% B, held for 2 minutes, then increased to 60% over 8 minutes, followed by a 1-minute wash at 90% B. Norfentanyl was not necessary to be chromatographically resolved from its ortho-, meta- and para-methyl acetyl isomers. To address co-elution with norfentanyl isomers, two highly selective transition ions unique to norfentanyl (m/z 233.2 → 177.1 and m/z 233.2 → 94.1) were employed. Fentanyl was monitored using transitions m/z 337.2 → 188.1 and m/z 337.2 → 281.2.

Results: The developed method demonstrated excellent sensitivity and specificity for both fentanyl and norfentanyl, effectively distinguishing them from the isomeric interferences. The assay's cutoff was set at 1.0 ng/mL, with an upper limit of linearity (ULOL) of 100 ng/mL. The limit of detection (LOD) was 0.25 ng/mL with the low QC (LQC) level at 0.4 ng/mL. No significant matrix effects were observed. An interference study at the LQC level, including opioid and benzodiazepine mixes and the three fentanyl isomers (**A**, **B** and **C**), showed no additional interference with norfentanyl—except in a sample containing acrylfentanyl (**D**).

Conclusion/Discussion: This validated LC-MS/MS method enables reliable quantification of fentanyl and norfentanyl in urine while effectively resolving interferences from newly reported isomeric analogs. The method circumvents interference of norfentanyl by **A**, **B**, and **C**, likely present as residual synthetic intermediates in commercial ortho-, meta-, and para-methyl acetyl fentanyl products. Compounds A, B and C are also highly likely the major human metabolites of ortho-, meta-, and para-methyl acetyl fentanyl in urine. Use of the widely utilized norfentanyl

transitions (m/z 233.2 → 84.1, m/z 233.2 → 55.1 and m/z 233.2 → 150.1) may lead to unresolved results or even false positives due to these interferences, underscoring the importance of ion selection in forensic toxicology LC-MS/MS assays.



P-025

Copper State Biosurveillance: Analyte Panel Extension for Detecting Drugs of Abuse in Urine by LC-HRAMS Using Residual Hospital Samples

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Abstract

Introduction: According to the Centers for Disease Control and Prevention (CDC), nearly 300 lives are lost daily in the United States due to drug overdoses. To reduce overdoses and health disparities, the CDC launched the Overdose Data to Action (OD2A) program in 2019. The Arizona State Public Health Laboratory (ASPHL) was awarded the OD2A grant in 2019 to assist with fatal overdose testing for county Medical Examiners. ASPHL has continued this work and has collaborated with several hospitals within Arizona to test non-fatal overdose samples for biosurveillance. As part of this OD2A grant work, in 2024 ASPHL developed and validated a method to detect drugs of abuse in urine. In 2025, ASPHL revalidated the method to add 24 additional analytes and update the instrument method based on lessons learned from a year of analytical work.

Objectives: The primary objective was to revalidate a qualitative method to identify drugs of abuse in human urine using liquid chromatography high-resolution accurate mass spectrometry (LC-HRAMS) with 24 additional analytes including several nitazenes. The secondary objective was to optimize the instrument method parameters based on insights from a year of running the method.

Methods: Human urine was digested with recombinant β-glucuronidase at room temperature, filtered through a β-glucuronidase removal plate using an automated liquid handler (Biotage Extrahera), and then diluted with water. In addition, isotopically labelled internal standards were added during sample preparation. Two glucuronidated compounds were included to monitor hydrolysis. The samples were analyzed on a Thermo Vanquish UHPLC coupled to either a Thermo QExactive HRAMS or Thermo Exploris 120 HRAMS. The LC method used an Agilent Poroshell 120 EC-C18, 2.1 x 100 mm, 1.9 µm column with a 12-minute gradient elution of Mobile Phases A (5 mM Ammonium Formate with 0.01% Formic Acid in Water) and B (0.01% Formic Acid in Acetonitrile). The MS method used full scan mode followed by data dependent fragmentation of drugs of abuse and their metabolites for a total of 67 analytes. Data was processed and evaluated using Thermo TraceFinder software.

Results: The validation data was evaluated by the ANSI/ASB Standard 036, First Edition 2019, Standard Practices for Method Validation in Forensic Toxicology. The method was evaluated for the following criteria: limit of detection, probability of detection, dilution integrity, specificity, carryover, ionization suppression or enhancement, interference, and uncertainty.

Discussion: The Arizona State Public Health Laboratory revalidated a qualitative LC-HRAMS method to detect drugs of abuse and their metabolites in urine with additional analytes. The LC gradient was changed in the revalidation to improve peak shape and retention of early eluting compounds. The MS2 fragmentation was changed from targeted fragmentation of every MS1 scan to a single data dependent fragmentation of each targeted peak, reducing redundant scans and increasing the number of MS1 scans across each peak. The method has been implemented for non-fatal overdose patient samples for the OD2A Strategy 4 grant work. The next step is to implement these changes for ASPHL's method for postmortem and antemortem blood samples submitted by medical examiners and hospitals to support OD2A Strategy 3 and 4, respectively.

P-026

Conversion of an Existing Norfentanyl Immunoassay to a Fentanyl Immunoassay

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Abstract

Introduction: In response to the Substance Abuse and Mental Health Services Administration's (SAMHSA) updated federal workplace drug testing guidelines, we've developed and received FDA 510(k) approval for a new fentanyl immunoassay. Effective July 7, 2025, SAMHSA will include fentanyl and norfentanyl in the federal workplace urine drug testing panel, establishing a 1 ng/mL cutoff concentration for both initial and confirmatory tests. Additionally, fentanyl screening immunoassays must demonstrate at least 5% cross-reactivity with norfentanyl.

Objectives: Our primary objective was to adapt a previously developed immunoassay, originally designed for norfentanyl with a 5 ng/mL cutoff, to meet SAMHSA's new 1 ng/mL fentanyl cutoff requirement, using fentanyl as the primary analyte.

Methods: The ***LZI Fentanyl III Enzyme Immunoassay*** is a homogeneous enzyme immunoassay utilizing ready-to-use liquid reagents. This assay operates on the principle of competition between the drug in the sample and an enzyme-labeled drug (glucose-6-phosphate dehydrogenase, G6PDH) for a fixed amount of antibody in the reagent. All performance characteristic studies were conducted on a Beckman Coulter AU5800 automated clinical chemistry analyzer.

Establishing a 1 ng/mL Fentanyl Cutoff

Reagents were carefully adjusted to generate a suitable curve for the qualitative analysis of fentanyl at a 1 ng/mL cutoff. Details regarding these changes, relative to the original norfentanyl assay, are documented in the 510(k) premarket submission, K251634, filed on June 18, 2025.

Precision and Reproducibility

Assay precision was evaluated following a modified Clinical and Laboratory Standards Institute (CLSI) EP05 protocol. Negative human urine was spiked with fentanyl to create samples at the 1 ng/mL cutoff concentration, as well as at ±25%, ±50%, ±75%, and ±100% of the cutoff. These samples were analyzed in duplicate across two runs per day over 22 days, totaling 88 replicates per concentration to assess within-run and total precision.

Method Comparison

To assess qualitative accuracy, 150 unaltered clinical urine samples were analyzed using the LZI Fentanyl III Immunoassay. Results were then compared to those obtained from a confirmatory liquid chromatography/mass spectrometry (LC/MS) method. Samples were sourced through collaborations with various clinical laboratories across the United States and Canada, including:

- APC Health (Tampa, Florida)
- Calgary Labs (Calgary, Canada)
- Carolina Liquid Chemistries Corporation (Greensboro, North Carolina)

- DTPM (Fort Payne, Alabama)
- Northwest Physicians Laboratories (Bellevue, Washington)
- Soloniuk Pain Clinic (Redding, California)
- Sterling Labs (Chicago, Illinois)
- TriCore Reference Laboratories (Santa Clara, California)
- University of California, San Francisco

Cross Reactivity

Fentanyl metabolites and structurally related compounds were individually spiked into negative urine pools. The lowest concentration of each compound producing a positive result equivalent to the fentanyl cutoff was determined to calculate the percent cross-reactivity.

Results: Qualitative precision results generally met expectations, with one exception: four of the 88 replicates (5%) at the 1 ng/mL concentration, which were expected to be positive, yielded negative results.

Among the 150 urine specimens analyzed in the method comparison:

- 35 drug-free urine samples tested negative by both immunoassay and LC/MS.
- 20 samples with fentanyl concentrations <0.5 ng/mL (by LC/MS) tested positive by immunoassay. These positive results were attributed to additional norfentanyl concentrations in these samples, ranging from 1.52 ng/mL to 39.86 ng/mL.
- 12 samples with fentanyl concentrations between 0.5 and <1.0 ng/mL (by LC/MS) tested positive by immunoassay. These positive results were attributed to additional norfentanyl levels in these samples, ranging from 0.48 ng/mL to 362.45 ng/mL.
- All 83 samples with fentanyl concentrations >1.0 ng/mL (by LC/MS) tested preliminary positive by immunoassay.

At the 1 ng/mL fentanyl cutoff, norfentanyl demonstrated 40% cross-reactivity, significantly exceeding SAMHSA's minimum requirement of 5%.

Discussion: The newly developed fentanyl immunoassay effectively meets SAMHSA's upcoming federal workplace testing criteria. The recent 510(k) approval confirms the assay's capability to detect fentanyl at the specified 1 ng/mL cutoff. The presence of norfentanyl in samples with low fentanyl concentrations provides a clear explanation for the positive results observed in cases where LC-MS/MS quantitation of fentanyl was below the assay cutoff. These findings collectively affirm the assay's suitability for routine federal workplace drug testing in accordance with the updated regulatory standards.

Conflict of Interest

All authors are employees of Lin-Zhi International, Inc.

P-027

Disappearing Delta-8 Carboxy-Tetrahydrocanabinol in Urine During Dilute and Shoot Quantification with Liquid Chromatography-Tandem Mass Spectrometry

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Abstract

Introduction: Cannabis is one of the most common substances (in addition to ethanol and other compounds) used at the US. Intensive quantitative methods have been established for the major psychoactive component of Δ9-THC, and its metabolite, Δ9-THC-COOH. Starting in 2020, the consumption of Δ8-THC increased significantly due to multiple reasons. To effectively quantify both Δ8-THC-COOH and Δ9-THC-COOH in urine, a low cost, high throughput test method was developed. A reduction in recovery for Δ8- and Δ9-THC-COOH was observed during the method development. Here we present our data demonstrating the lower recovery of Δ8-THC-COOH during the sample preparation process.

Objectives: Quantitation of Δ8-THC-COOH from urine and to determine the cause of the Δ8-THC-COOH loss.

Methods: The 33 Δ8-THC-COOH samples that screened positive via immunoassay were prepared and hydrolysis with sodium hydroxide in a glass tube, and the concentrations were measured on our newly developed. validated dilute-and-shoot LC-MS/MS method. Agilent 6495 Mass Spectrometer coupled with a StreamSelect LC system and CORTECS T3 analytical column (2.1 x50 mm, 1.6µm) were used. The hydrolysis, extraction process, and the column are all handled in room temperature.

Results: Compared to the screening results, the concentrations generated from LC-MS/MS were lower in all the samples, and on average the analyte losses were 30.5%, indicating that the analyte loss occurred when samples were transferred.

To test the time for analyte degradation, 10 random positive samples were aliquoted to glass tubes and measured at predetermined time intervals. After aliquoting, significant reduction was observed from 1 to 3 hours (over 20%), but there were no significant recovery differences from 3 to 24 hours---for this time period, there was only a slight reduction in calculated concentration (~5%).

Following this observation, tubes made from different materials were tested. The same volume of specimen was aliquoted into glass and plastic tubes, and the analytes concentrations were measured at 3, 6, 12 and 24 hours. At each time point, there was no concentration difference between the two types of tubes, contradicting to the previous reports that more THC is lost in plastic tubes.

Finally, the surface-to-volume ratio was tested. The specimens were aliquoted into 5 mL of glass tubes with volumes of 0.5 mL, 1 mL, 2mL and greater than 3 mL, and then quantified after 24 hours. The most analyte loss was found in the 0.5 mL test tube, which had the highest surface-to-volume ratio.

Discussion: With less side-affects, the Δ8-THC is considered a “nicer young sibling of Δ9-THC”. The Δ8-THC that naturally occurs in the marijuana plant is very low; thus, the detectable Δ8-THC predominantly comes from synthetic products. With marijuana usage, the prevalence of Δ8-THC is about 10-35%. To accurately quantitate the Δ8-THC-COOH, minimizing the processing steps to reduce the analyte loss is the key. Our data shows that the analyte loss occurs during specimen transfer, no matter what material is used, and the most loss occurs during the first three hours. In addition, the surface-to-volume ratio plays an important role in analyte loss. This data provides fundamental information for the accurate detection of Δ8-THC-COOH. The limitation of this study is that, only Δ8-THC-COOH was monitored. Testing both Δ8-and Δ9-THC COOH for analyte loss at the same time will provide further useful information and will require future investigation.

P-028

Optimizing Hair Decontamination for Cocaine: Evaluating Solvents, Wash Frequency, and Agitation Methods

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Abstract

Introduction: Hair toxicology is a widely used tool to assess drug use patterns over extended periods. However, differentiating external contamination from actual drug incorporation remains a major interpretative challenge, especially with substances like cocaine. The Society of Hair Testing (SoHT) recommends standardized washing procedures to remove external contamination prior to analysis, but there is currently no consensus on the most effective method. This study evaluates various decontamination strategies to identify optimal conditions for removing externally deposited cocaine from hair samples.

Objectives: To investigate the influence of organic solvent type, number of washes, and agitation mode on the effectiveness of cocaine decontamination in hair, with the goal of identifying the most reliable protocol for external contamination removal.

Methods: Hair samples were artificially contaminated using four different conditions: soaking in 0.1 (low concentration) or 1.0 µg/mL (high concentration) of cocaine-d_3 for 1 or 24 hours. Three organic solvents - dichloromethane (DCM), acetone, and ethyl acetate (EtAc) - were tested, and washing frequency was varied from one to five washes. Samples were subjected to either vortexing or ultrasonic agitation (2 min each). Cocaine-d_3 residue was extracted and quantified by LC-MS/MS. Each variable was systematically evaluated to assess its impact on decontamination efficiency.

Results: Dichloromethane yielded inconsistent results with concentrations both decreasing and increasing with sequential washes. Acetone and ethyl acetate demonstrated progressive decreases in cocaine-d_3 concentrations across sequential washes of up to 25 times less concentration after wash number five. Concentrations remained relatively stable after the third wash and three sequential washes proved more effective than one or two with a decrease of 1.5, 1.6 and 2.0 times less cocaine-d_3 after 1, 2 and 3 washes, respectively. Variability between the first and second wash was occasionally observed for acetone with increases of up to 1.2 times more cocaine-d_3 than the previous wash. Agitation by vortexing slightly outperformed ultrasonic treatment in most scenarios, with 2 times less drug versus 1.2 times, respectively. Overall, the best conditions were a combination of an aqueous wash, followed by two methanolic washes and finally three ethyl acetate washes (each with 2 min vortex). Under these conditions, a decrease of up to 7 times less cocaine-d_3 was observed for 1mg/mL contamination for 24h.

Discussion: Ethyl acetate, applied in triplicate washes with vortexing, is the most effective decontamination strategy identified in this study for removing externally deposited cocaine from hair, when preceded with an aqueous wash and two methanolic washes (with a 2min vortexing step each). These results inform the development of standardized protocols to differentiate contamination from true drug incorporation. Future work will apply this method to authentic user samples and assess performance across various hair types (e.g., bleached, dyed, or different ethnic backgrounds). This work contributes to improving the accuracy and interpretability of hair toxicology in forensic contexts.

P-029

Quantitative Analysis of Δ8- and Δ9-THC and their Main Oxidative Metabolites in Urine by GC/MS and GC-MS/MS

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Abstract

Introduction: Cannabis abuse has escalated significantly in Japan in recent years, particular among younger generations, prompting stricter legal measures and heightened public concern. In response to the growing issue, Japan revised its cannabis laws in December 2024. Under the previous Cannabis Control Act, only possession and cultivation – but not use – of cannabis were illegal. The revised law criminalizes cannabis use and legalizes medical products derived from cannabis; this legal reform creates a demand for reliable analytical methods to detect cannabis use.

Since tetrahydrocannabinol (Δ^9-THC) is metabolized to 11-hydroxy-Δ^9-THC and Δ^9-THC-COOH, it is important to have a method that can reliably detect both the parent compound and its metabolites in urine for proof of use. It is also important to distinguish positional isomers of these compounds, especially Δ^8-THC and its metabolites, as Δ^8-THC is also illegal in Japan. While LC-MS/MS has become the more commonly used method for detecting THC metabolites in urine, gas chromatography-mass spectrometry (GC/MS) and GC-tandem mass spectrometry (GC-MS/MS) are still widely used in many forensic laboratories due to their robustness and good sensitivity, and ability to separate positional isomers. This is particularly relevant for 11-hydroxy-metabolites of Δ^8- and Δ^9-THC, which is often difficult to resolve using LC-MS/MS.

Objectives: The primary aim of this study was to establish a reliable and robust GC-MS(/MS) method for the simultaneous detection of Δ^8-THC, Δ^9-THC, and their main oxidative metabolites in urine.

Methods: Reference and deuterated internal standards (IS) for Δ^8-THC, Δ^9-THC, 11-OH-Δ^8-THC, 11-OH-Δ^9-THC, Δ^8-THC-COOH, and Δ^9-THC-COOH were either purchased from Cayman Chemical (Ann Arbor, Michigan, USA) or synthesized in-house. Blank urine (200 µL) was spiked with target and IS compounds (50 ng/mL), followed by alkaline hydrolysis. Hydrolyzed urine underwent liquid-liquid extraction (LLE) using hexane:ethyl acetate (7:1). Extracts were dried under nitrogen, reconstituted in 25 µL of acetonitrile, mixed with 25 µL of BSTFA (1% TCMS), and heated at 70°C for 30 minutes for TMS derivatization.

Analysis was performed on a GCMS-TQ8040 NX triple quadrupole mass spectrometer (Shimadzu Corporation, Kyoto, Japan) with SH-I-5Sil MS column (30 m, 0.25 mm I.D., 0.25 µm film thickness). The column oven temperature program was as follows: initial temperature 150°C held for 1 minute, followed by a 15°C/min ramp to 320°C, with a final hold for 3 minutes. The inlet temperature was set at 280°C, and the injection volume was 2 µL in the splitless mode. The MS detector settings included an interface temperature of 280°C and an ion-source temperature of 230°C. GC-EI-MS(/MS) analyses were conducted in either scan mode (*m/z* 45-600) or MRM mode.

Results: The parent and oxidative metabolites of the Δ^8- and Δ^9-THC isomers were successfully separated and chromatographically resolved. Linearity was demonstrated for all analytes over

the calibration range of 1-500 ng/mL (R>0.995). Inter-day and intra-day accuracy (n=5) for low (15 ng/mL) and high (250 ng/mL) concentrations ranged from 95-113% in scan mode and 102-106% in MRM mode. Precision was within 5% for all analytes in both inter- and intra-day measurements. The LLOQ was determined to be 10 ng/mL for GC/MS scan and 1 ng/mL for GC-MS/MS for all analytes.

Discussion: A robust LLE and GC-MS(/MS) method was developed for the simultaneous analysis of Δ^8-THC, Δ^9-THC, and their oxidative metabolites in urine. GC/MS-scan analysis provides full-spectrum confirmation of the target analytes and demonstrated quantitative accuracy sufficient to detect them at low ng/mL concentrations in urine. The GC-MS/MS method exhibited sensitivity down to 1 ng/mL, suitable for confirming cannabis used under the revised Japanese law.

P-030

Performance Evaluation of Antibody and Antigen Conjugate Pair for Detection of Xylazine and its Metabolite Hydroxy Xylazine in Lateral Flow Assay

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Abstract

Introduction: Xylazine is a veterinary tranquilizer and an alpha-2 adrenergic receptor agonist that is increasingly misused in combination with synthetic opioids, especially in the United States, where it has been associated with numerous overdose deaths. Detection of xylazine is critical because its effects cannot be reversed with naloxone, the standard treatment for opioid overdoses. In preclinical settings, alpha-2 adrenergic receptor antagonists have been researched as treatment option for xylazine overdose.

Xylazine and its hydroxy metabolite can be detected from blood and urine samples of opioid users. Among the various analytical methods available, lateral flow assays remain a key approach for detecting drugs of abuse use in urine. The sensitivity of these assays is highly dependent on the optimal pairing of antibody and antigen conjugate.

Medix Biochemica develops antibodies, antigens, and other critical raw materials for in vitro diagnostics (IVD) tests.

Objectives: The aim of the study was to develop antibodies for the detection of xylazine and its main urine-excreted metabolite, hydroxy-xylazine, intended for use by diagnostic test developers in xylazine IVD test. To complement the monoclonal antibodies in a competitive assay format, we also developed xylazine-hapten conjugates with varying conjugation ratios.

Methods: To evaluate sensitivity of the mAb and conjugate pairs, the BSA-conjugates were labelled with a fluorescent reporter and studied in a competitive assay format with free hydroxy-zylazine. The most sensitive pairs were further evaluated in lateral flow.

Results: We have previously observed that the conjugation ratio can significantly affect assay sensitivity and potentially specificity. Similar results were observed in this case, too.

Discussion: In this poster, we present data demonstrating how the developed monoclonal antibodies and antigen conjugates perform together in lateral flow format. This pre-screening approach can support diagnostic test developers in selecting suitable components for assay development.

Conflict of Interest

Salary from Medix Biochemica

P-031

Can Desloratadine Overdose Result in Death?

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Abstract

Introduction: Allergic diseases are recognized as civilization-associated conditions, with an estimated global prevalence of approximately 18%, including up to 25% of the Polish population. Current therapeutic strategies for allergic rhinitis (AR) predominantly include the use of intranasal glucocorticosteroids and second-generation antihistamines. A representative example is desloratadine, which exhibits markedly greater potency than many other antihistamines used in AR therapy. Desloratadine is characterized by a favorable safety profile and, in pediatric populations up to 5 years of age, is administered at a dose of 1.25 mg once daily. The patient, a male child of Polish heritage born at 34 weeks of gestation, was evaluated at birth as being in moderate conditions. At the age of 2.5 years, he was placed in institutional foster care. During his stay, the child experienced recurrent episodes of airway obturation, which were identified as having a multifactorial etiology, including anatomical, functional, developmental factors and other contributing conditions (including bronchial asthma, AR, lack of prior maternal awareness regarding the child's health issues). After three months in the foster care facility, desloratadine was introduced into the therapeutic regimen for AR administered at the recommended dose. The child's death occurred suddenly during the sixth month of residence in the facility.

Objectives: The aim of the presentation is to showcase the results of the analysis of biological material obtained from the body of a child, in whom there was a suspicion of an overdose of a medication containing desloratadine.

Methods: Blood and urine were analyzed for a wide range of xenobiotics with various effects on the human body, including medications, narcotics, and new psychoactive substances, using GC-MS, HPLC-DAD, LC-MS/MS, and LC-Q/TOF methods. For this purpose, blood and urine were extracted using the liquid-liquid extraction method. Additionally, the urine was subjected to derivatization to convert the present xenobiotics into silyl derivatives. No enzymatic hydrolysis was performed prior to the analysis.

Results: Analytical testing revealed the presence of desloratadine in both blood and urine samples, with measured concentrations of 112 ng/mL and 270 ng/mL, respectively. Additionally, its primary metabolite, 3-hydroxydesloratadine, was detected at concentrations of 16 ng/mL in blood and 280 ng/mL in urine. Subtherapeutic levels of ibuprofen were also identified.

Discussion: The therapeutic concentration range of desloratadine measured in living individuals is 2–7 ng/mL in the blood of adults, while in children up to 5 years of age it does not exceed 3 ng/mL. Desloratadine is primarily metabolized in the liver via hydroxylation by the cytochrome P450 enzyme CYP2C8, followed by glucuronidation. Approximately 6% of individuals (both adults and children) exhibit slow metabolism, rendering them susceptible to increased exposure to desloratadine. In such individuals, the biological half-life (T½) exceeds

50 hours, in contrast to normal metabolizers, whose T½ is 27 hours. Clinical studies have administered doses up to nine times higher than the therapeutic dose without observing significant adverse effects. In this case, the low blood level of 3-hydroxydesloratadine may suggest impaired desloratadine metabolism; however, this observation alone is not sufficient to definitively classify the individual as a slow metabolizer. Other factors, such as individual variability in enzyme activity or postmortem metabolic processes could also contribute to this finding. It should be strongly emphasized that such a high concentration of desloratadine in blood has not been previously reported in the scientific literature, and, at the same time, there is a lack of medical evidence to associate it with the cause of death in the presence of other coexisting risk factors. The cause of death was determined to be respiratory arrest resulting from critical airway obstruction in a child with multiple risk factors.

P-032

Trends in Tranq 2: The Emergence of Medetomidine with Xylazine in Oral Fluid Toxicology Cases in the American Great Lakes Region

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Abstract

Introduction: Medetomidine (which goes by the street name "Rhino Tranq") is an α-2 adrenergic agonist that is increasing in prevalence in illicit drug markets in the United States. Like its predecessor xylazine, medetomidine has a similar mechanism of action with a reported greater degree of potency and is also largely found alongside illicit fentanyl. Medetomidine was recently added to our Liquid chromatography tandem mass spectrometry (LC-MS-MS) confirmation methods, and here we present our findings with this substance alongside updated xylazine trends among the health and human services agencies and court systems we work with in Michigan, Ohio, Indiana, Illinois and Wisconsin.

Objectives: Our objective was to investigate trends in medetomidine and xylazine use from oral fluid samples collected in the Great Lakes states from March 2023 through May 2025.

Methods: Oral fluid specimens were analyzed following a screen-to-confirm workflow using a combination of ELISA and LC–MS-MS methods at Forensic Fluids Laboratories. We focused on the incidence and spread of medetomidine and xylazine (LOD 0.1 and 1 ng/ml respectively) among recreational drug users by using samples that screened and confirmed positive for at least one of the following substances: methamphetamine, cocaine, opiates, and fentanyl (n = 86,093). Plots and maps were generated using NumPy, Pandas, Matplotlib and GeoPandas libraries in Python.

Results: Xylazine and medetomidine monitoring began in March of 2023 and August of 2024, respectively. In the time since we began monitoring these substances, the prevalence of each of these has increased. In over 95% of xylazine or medetomidine positive cases (3,001 and 536 total cases respectively), fentanyl was also reported in the sample. Over three quarters of xylazine and medetomidine cases were also reported with multiple drug classes. Focusing specifically on fentanyl positive cases, xylazine was initially found in approximately one in every five of fentanyl positive samples and as of February 2025 was found in one in every three. In ten months, medetomidine-positive fentanyl cases increased from one in twenty to one in three. The overall xylazine state prevalence decreased in Michigan (3.17% - 2.10%), Indiana (3.96% - 2.07%), and Wisconsin (2.99% - 1.05%) from the first nine-month study period to the last nine-month period. Medetomidine, on the other hand increased in prevalence in each of these states (0.41% - 1.59%, 0.85% - 1.92%, 0% - 1.10% for Michigan, Indiana, and Wisconsin respectively). For Illinois and Ohio, the state prevalence of xylazine and medetomidine both increased (Illinois – 0.84% - 1.69%, 0.14% - 0.27%, Ohio – 4.22% - 4.29%, 2.44% - 3.23% for xylazine and medetomidine, respectively).

Discussion: The sharp rise in fentanyl cases containing medetomidine over the first seven months of monitoring indicates a rapid adoption of this substance into illicit fentanyl markets in the Great Lakes states. Xylazine, on the other hand, increased in fentanyl cases over the first year, but has generally plateaued in this region in recent months. In addition, the overall xylazine prevalence decreased from the first year to the second for most states we work in, while medetomidine prevalence increased from the first three months to the last four months. Taken together, these

data suggest an active evolution in fentanyl drug trends taking place in Great Lakes states, possibly showing early signs of medetomidine replacing xylazine in illicit fentanyl in the future.

Conflict of Interest

2) I am a paid salary employee of Forensic Fluids Laboratories, who are 4) covering my travel expenses to SOFT

P-033

Evaluation of Phosphatidylethanol (PEth) as a Biomarker for Alcohol Use in Decomposed Postmortem Blood Samples

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Abstract

Introduction: Phosphatidylethanol (PEth) is a direct and specific biomarker of ethanol consumption, formed in cell membranes by the action of phospholipase D in the presence of ethanol. Unlike traditional biomarkers, PEth reflects repeated or chronic alcohol intake over days to weeks, making it valuable in both forensic and clinical toxicology. Due to its high specificity and extended detection window compared to blood ethanol and other volatile markers, PEth has become a reliable indicator of alcohol consumption.

In postmortem toxicology, ethanol interpretation is often confounded by microbial fermentation and endogenous production, especially in decomposed bodies. PEth provides a advantage, as it is not formed postmortem and may remain stable in preserved tissues. Antemortem interpretation thresholds have been proposed concentrations <20 ng/mL indicate abstinence or incidental exposure, 20–200 ng/mL suggest moderate or occasional use, and values >200 ng/mL indicate chronic or excessive alcohol consumption.

Objectives: This study evaluated the utility of PEth metabolites 16:0/18:1 and 16:0/18:2 in detecting alcohol use in postmortem whole blood in decomposed cases.

Methods: PEth concentrations were quantified in 53 postmortem cases showing decomposition. Of the 53 cases, 23 involved samples collected from multiple sites, resulting in a total of 76 samples. Blood samples were processed using a modified published method. Briefly, calibrators, controls, and case samples were extracted using a Biotage ISOLUTE® SLE+ plate, and analyzed on a Waters TQ-S micro LC-MS/MS system with a Luna Phenyl-Hexyl column The method was validated following ANSI/ASB Standard 036.

Results: All samples were taken from cases where decomposition was noted and all samples appeared as dark red liquid. Postmortem bloods were labeled as inferior vena cava (IVC, n=15), heart (n=31), iliac vein (n=6), chest cavity fluid (n=17), decomposed samples (n=6), and one additional “pooled” sample. Based on the proposed clinical interpretation, in 13 cases with both IVC (0-1221 ng/mL 16:0/18:1 & 6-470 ng/mL 16:0/18:2) and heart blood (0-1563 ng/mL 16:0/18:1 & 0-635 ng/mL 16:0/18:2), five cases indicated heavy drinking, three moderate drinking and two incidental use. Four cases showed discrepancies, two between heavy and moderate drinking (-114 and 111 % difference IVC to heart), and two between moderate and incidental use (73 and 60 % difference IVC to heart). In two cases comparing heart (25 and 22 ng/mL 16:0/18:1 & 34 and 68 ng/mL 16:0/18:2) and chest cavity fluid (21 and 13 ng/mL 16:0/18:1 & 9 and 6 ng/mL 16:0/18:2),

one pair showed disagreement, with the heart sample suggesting moderate use. Among 15 chest cavity fluid (334-0 ng/mL 16:0/18:1 & 597-0 ng/mL 16:0/18:2), 13 indicated incidental exposure, one showed moderate use, and one suggested heavy drinking. Of the six decomposed/pooled samples, five indicated incidental exposure. One matched IVC sample (39 ng/mL 16:0/18:1 & 115 ng/mL 16:0/18:2) showed moderate use. In six cases comparing heart (33-1271 ng/mL 16:0/18:1 & 808-43 ng/mL 16:0/18:2) to lilac vein (19-223 ng/mL 16:0/18:1 & 23-538 ng/mL 16:0/18:2) one pair showed discrepancies (79 % difference heart to lilac) between heavy drinking and moderate use and one pair showed discrepancies (54 % difference heart to lilac) between moderate use and incidental exposure. Two cases agreed on heavy drinking and two case agreed on moderate uses. Independent analysis of heart and iliac samples found seven indicating heavy drinking, two moderate use, and one incidental exposure.

Discussion: PEth was detectable in decomposed postmortem blood samples, but interpretation was complicated by inconsistencies between anatomical sites and quality of the “blood” from these samples. These findings raise concerns about PEth’s postmortem stability and reliability, particularly when concentrations are below the 200 ng/mL threshold. While PEth ≥200 ng/mL may indicator further investigation of chronic use, all PEth results should be interpreted with caution in decomposed cases.

Acknowledgments

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Keywords

Phosphatidylethanol, PEth, Postmortem Toxicology, Decomposition, Ethanol Biomarkers

P-034

Cross-Reactivity Assessment of a Highly Sensitive Polyclonal Antibody for the Detection of Multiple Nitazene Analogues

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Abstract

Introduction: Synthetic opioids are one of the fastest growing new psychoactive substances (NPS) subclasses and have been implicated in multiple cases of severe toxicity and fatalities. Following an international drive to limit the distribution of fentanyl analogues other non-fentanyl opioids have become more prevalent in the NPS opioid market [1]. Nitazenes, originally synthesized and investigated in the 1950's and 60's, are a class of synthetic opioids that have emerged on the illicit drug market being sold in different forms including powders, tablets, sprays and vaping liquids [2]. Nitazene analogues can be highly toxic through high binding to the mu-opioid receptors. This can lead to adverse sedation and respiratory failure, with nitazene related deaths being reported internationally, including the USA and Canada. [3] Randox have developed an antibody for the detection of nitazenes.

Objectives: To determine the detection of various nitazene compounds by employing a competitive Enzyme Linked Immunosorbent Assay (ELISA) technique as a screening tool for assessment of a polyclonal antibody.

Methods: A competitive ELISA was used for the screening of various nitazene compound on the polyclonal antibody of interest. The capture antibody was immobilised on a 96-well microtiter plate for assessment. A serial dilution of Isotonitazene antigen was prepared with a top level of 1 ng/ml and applied to the coated plate as the standardizing antigen. The various nitazene compounds were prepared as individual dilution series in buffer with a top level of 1 ng/ml to be assessed for potential cross reactivity and applied to the coated plate. A specific Horseradish Peroxide (HRP) labelled tracer is added to each well of the microtiter plate, the labelled tracer competes with the standardizing antigen/cross reacting compound for binding to the limited number of antibody sites available during the incubation period. A wash step is completed to remove any unbound material and enzyme substrate is added to initiate a colorimetric reaction. After 20 minutes the reaction is stopped with an acidic solution to stabilise the colour change for accurate measurement of the optical density with absorbances being read at 450 nm. The colour intensity is inversely proportional to the concentration of the target antigen in the sample. The optical density readings are used to construct a standard curve.

Results: The IC50 was calculated for the standardizing antigen, isotonitazene, and for each compound being tested for cross reactivity. The IC50 value is used to calculate the approximate percentage cross reactivity. The results are as follows: isotonitazene 100%, etodesnitazene <10%, protonitazene 34%, N-pyrrolidino etonitazene 69%, 5-aminisotonitazene <10%, metonitazene 77%, butonitazene 47%, etonitazene 63%, clonitazene 63%, N-pyrrolidino metonitazene 75%, N-desethyl isotonitazene 35%, N-pyrrolidino protonitazene 37%.

Discussion: With the reported occurrences of nitazene related overdoses this antibody can act as a useful tool for immunoassay-based screening. With the sensitive detection of a wide range of nitazene compounds it has the potential to be used in a variety of screening applications including

ELISA and Biochip Array Technology (BAT) which can be utilized on semi-automated and fully automated analysers to provide preliminary analytical test results for multiple forensic matrices.

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Conflict of Interest

Salary

P-035

The Line Goes Up?: Examining Fentanyl Concentrations Over the Years

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Abstract

Introduction: Since fentanyl's emergence in the illicit drug supply in the early 2010s, concentrations in both antemortem and postmortem casework have increased. Fentanyl is the primary opioid detected in opioid-related deaths and the concentrations in human performance and postmortem casework overlap. Understanding the trend of fentanyl concentrations is important for public health programs such as naloxone access.

Objectives: The casework sent to the lab is from many different states and regions across the United States. Data for fentanyl was reviewed January 2012 – May 2025 (n=42,331) to examine the trends of postmortem and antemortem concentrations. Antemortem (Group 1) is blood specimens marked hospital, ER, serum, or whole blood. Peripheral (Group 2) is femoral or peripheral where peripheral is noted as such by the investigator on the submission form. Between Peripheral and Central (Group 3) is blood if marked as subclavian, iliac, carotid, or jugular. Central (Group 4) is blood or fluid collected from central, abdominal, heart, IVC, SVC, aorta, cavity, pleural, cardiac, or spleen. Uncategorized (Group 5) is arterial, autopsy, blood clot, inguinal, mixed, not indicated, or subdural blood.

Methods: Blood specimens are shipped to the lab in tubes containing preservative, typically gray or lavender top tubes. They are extracted using a cold acetonitrile extraction containing deuterated internal standard. Prior to 2020, the screen was performed using liquid chromatography paired with triple quadrupole mass spectrometry (LC-MS/MS). At the beginning of 2020, the screening method used liquid chromatography paired with quadrupole time of flight mass spectrometry. Confirmation testing is done with LC-MS/MS. Prior to 2020, the screening and confirmation cutoffs for fentanyl were 5 ng/mL and 0.5 ng/mL respectively. Starting in 2020, the cutoffs were changed to 0.2 ng/mL and 0.1 ng/mL respectively. All methods were validated following ABFT requirements, and ASB guidelines and standards.

Results: Averages and medians were calculated for each group across its entire data set. Trend lines were added to see which direction the data moved from 2012 to 2025. Groups 2-5 all show upward trends for their respective average and median. Group 1's average shows an upward trend and the median shows a slight downward trend. Group 1 has an average and median of 6.9 ng/mL and 3.7 ng/mL respectively. Group 2 has an average and median of 15.2 ng/mL and 8.1 ng/mL respectively. Group 3 has an average and median of 24.1 ng/mL and 11.9 ng/mL respectively. Group 4 has an average and median of 22.2 ng/mL and 10.5 ng/mL respectively. Group 5 has an average and median of 20.2 ng/mL and 10.1 ng/mL respectively.

Discussion: The lab primarily deals with postmortem casework. The antemortem specimens are cases where an individual was brought to the hospital and died sometime after admission. The overall data trends show that antemortem concentrations from Group 1 are lower than postmortem concentrations from Groups 2-5. This would be supported by accounting for postmortem redistribution (PMR). Another possibility is that individuals experiencing toxic effects at lower doses may be able to undergo resuscitative efforts at the hospital. Groups 3 & 4 are higher than Groups 1 & 2 which is also explainable by PMR. This study does not account for

medically administered fentanyl for cases where trauma was sustained. In these cases, fentanyl concentrations are typically lower than overdose cases. A rise in concentration of postmortem fentanyl could indicate an increase in tolerance at the population level and/or more potent product available. The rise in fentanyl concentrations would necessitate the use of more naloxone over a greater time frame to block the fentanyl effects.

Reviews

Additional comments will be addressed in final presentation.

P-036

Semi-Quantitative Simultaneous Screening of Multiple Drugs of Abuse from a Single Urine Sample on the New Random Access Biochip Technology Analyzer (RABTA)

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Abstract

Introduction: In the ever-evolving field of toxicology and drug screening, speed, efficiency and automation are critical. The RABTA is a new Randox analytical instrument offering versatility to transform the screening process for drugs of abuse. A single sample can be screened for multiple drugs of abuse, with results reported in just over 36 minutes, and additional sample results reported every minute after that. The analyzer allows multiple arrays to be ran at the same time with continuous loading and random-access capabilities, allowing workload to be prioritized. RABTA is a semi-quantitative screening platform that provides a preliminary result for multiple drugs of abuse and/or their metabolites, from a single urine sample.

Objectives: A new biochip array has been developed for use with the novel RABTA which is capable of simultaneously screening 14 drugs of abuse and their markers in urine. All assays are listed with their cut offs; 6-Monoacetylmorphine (10ng/mL), AB-PINACA (10ng/mL), Amphetamine (500ng/mL), Buprenorphine (5ng/mL), Benzoylecgonine (150ng/mL), Ethyl Glucuronide (1000ng/mL), JWH018 (20ng/mL), Methamphetamine (500ng/mL), MDMA (500ng/mL), Opiates (2000ng/mL) [2] , Oxazepam (300ng/mL), Phenobarbital (200g/mL), THC ((-)-11-nor-9-Carboxy-Δ 9-THC) (50ng/mL), UR144 (20ng/mL) and Creatinine as an adulteration marker.

Methods: The new biochip array was applied to the RABTA to assess performance. Precision and repeatability of the 15 drugs of abuse were assessed by running 10 replicates of 9 point calibration curves, spiked with each drug of abuse. Additionally, the Limit of Detection (LOD) was completed to determine the sensitivity of each assay, in which 20 negative urine samples were assessed.

Results: The average precision of each assay is as follows; 6MAM 12%, AB-PINACA 9%, Amphetamine 9%, Phenobarbital 10%, Buprenorphine 9%, Benzoylecgonine (Cocaine) 6%, Creatinine 10%, Ethyl Glucuronide 17%, Oxazepam 7%, Methamphetamine 7%, UR144 6%, JWH018 9%, MDMA 7%, THC 5% and Opiates 8%.

The sensitivity results are as follows; 6MAM ≤2.5ng/mL, AB-PINACA ≤0.6ng/mL, Amphetamine ≤15.7ng/mL, Phenobarbital ≤50ng/mL, Buprenorphine ≤1.67ng/mL, Benzoylecgonine (Cocaine) ≤37.5ng/mL, Ethyl Glucuronide ≤250ng/mL, Oxazepam ≤75ng/mL, Methamphetamine ≤14.5ng/mL, UR144 ≤1ng/mL, JWH018 ≤6.7ng/mL, MDMA ≤16.7ng/mL, THC ≤5.6ng/mL, OPIAT ≤170ng/mL.

Discussion: The array demonstrated a high level of precision and reproducibility on the RABTA. Furthermore, all assays met sensitivity targets. Results show the capability of the RABTA to detect a wide range of classic and novel synthetic drugs from a single urine sample. As patterns of drug use continue to evolve and novel substances emerge, the demand for accurate, efficient and adaptable drug testing in forensic settings remains critical [1].

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2. SAMHSA Guidelines

Conflict of Interest

Salary

P-037

A Case Report Involving Fatal MDPiHP Intoxication

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Abstract

Introduction: Synthetic cathinones are phenethylamine derivatives designed to mimic cathinone, the naturally occurring alkaloid found in the *Catha edulis* plant leaves. These compounds exhibit psychostimulant effects comparable to cocaine, amphetamine, and methylenedioxymethamphetamine. New analogues often consumed as legal alternatives largely due to deliberate structural modifications that allow the circumvention of existing drug-specific synthetic cathinone scheduling. As a result, synthetic cathinones constituted the second most representative class of novel psychoactive substances (NPS) in 2024, with over 100 identified analogues in the global illicit market.

Among these NPS, α-pyrrolidinophenones, such as 3,4-methylenedioxy-α-pyrrolidinohexanophenone (MDPHP), have become increasingly implicated in forensic toxicology casework due to high potency. In contrast, MDPHP's structural isomer, 3,4-methylenedioxy-α-pyrrolidinoisohexanophenone (MDPiHP) remains poorly characterized. Apart from the initial report of MDPiHP in Sweden by the European Union Drugs Agency (EUDA) in 2020, and information from the manufacturer of its analytical reference standard, there are no additional data available on MDPiHP. This poster presents a postmortem case involving MDPiHP in the United States.

Objectives: To increase awareness of novel synthetic cathinone isomer MDPiHP and its concentration in postmortem blood.

Methods: The University of Florida Forensic Toxicology Laboratory received a heart blood sample from an investigation involving a male inmate found unresponsive in his cell and later pronounced dead following unsuccessful resuscitation efforts. A search of his jail cell yielded an unknown substance. The decedent had a known history of cardiac disease, and methamphetamine had been found in his possession one week prior to death.

MDPiHP was presumptively identified through initial testing utilizing gas chromatography-nitrogen phosphorus detection (GC-NPD) and gas chromatography-mass spectrometry (GC-MS). An aliquot of blood was forwarded to the Center for Forensic Science Research and Education (CFSRE) Toxicology Laboratory for confirmation and quantitation.

Screening was performed using a SCIEX X500R liquid chromatograph/quadrupole time-of-flight mass spectrometer (LC-QTOF/MS) with SWATH acquisition. Analysis was conducted via a four-point standard addition, which included one unfortified aliquot ("blank") and three aliquots up-spiked to final concentrations of 0.2, 2.0 and 20 ng/mL. Fentanyl-D_5 (10 ng/mL) was added as the internal standard to all samples. Samples were extracted by liquid-liquid extraction using 0.1 M borax buffer (pH 10.4) and 70:30 N-butyl chloride: ethyl acetate. Quantitation was achieved on a Waters® Xevo-TQS® micro liquid chromatograph/tandem mass spectrometer (LC-MS/MS). The LC-MS/MS method had previously been optimized to chromatographically separate MDPHP and MDPiHP.

Results: MDPiHP was definitely identified in heart blood at a concentration of 550 ng/mL. Naloxone was also qualitatively detected in the heart blood. The medical examiner certified the cause of death as MDPiHP intoxication and the manner of death as accidental.

Discussion: This report describes the first documented postmortem case where the structural isomer MDPiHP was confirmed and analytically differentiated from the more recently emerging MDPHP. Owing to their close structural similarity, it may be reasonable to assume that both compounds elicit similar pharmacological effects. However, despite the increasing detection of MDPHP in forensic casework globally, published data on its pharmacology, toxicity and prevalence in casework remains limited, with even lesser known about MDPiHP. The identification of MDPiHP highlights the importance of distinguishing between closely related isomeric compounds to mitigate misidentification and misreporting in routine toxicological analyses and support accurate forensic surveillance amid the rapidly evolving NPS drug landscape.

P-038

Concentrations of Fentanyl in Hair Collected for Court-Ordered Mandatory Drug Testing

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Abstract

Introduction: Hair testing provides a historical record of drug use, detecting substances ingested weeks to months prior. As a result, hair testing is increasingly used in legal settings to monitor adherence to court-ordered treatment programs, probation or parole conditions, or parental custody arrangements. Fentanyl is a significant contributor to the rising rates of opioid overdose deaths and drug-related fatalities and reports of fentanyl by forensic laboratories have increased rapidly since 2015. However, many current court-ordered mandatory drug testing (COMDT) programs still do not include fentanyl in their routine testing panels.

Objectives: The goal of this study was to determine the prevalence of fentanyl in hair specimens collected for COMDT and provide data on fentanyl and norfentanyl concentrations to inform cutoff decisions for programs adding fentanyl to their testing panels.

Methods: We analyzed 1025 hair specimens, originally collected for COMDT, for 26 substances including 13 fentanyl-related compounds. All specimens were collected in the United States between November 2020, and February 2021. Hair specimens were decontaminated with serial washes with water, methanol, and methylene chloride then pulverized and extracted overnight using methanol. Supernatants underwent SPE and were analyzed using an ASB validated LC-MS/MS method for which precision, bias, LOD, LLOQ, carryover, matrix effects, stability, and interference were assessed.

Results: Methamphetamine was the most frequently detected compound (n=266, 26%), followed by hydrocodone (n=157, 15%). Fentanyl was the most detected fentanyl-related compound, followed by 4-ANPP, which is expected since 4-ANPP is both a fentanyl metabolite and impurity. Fentanyl was detected in 151 (15%) hair specimens. 12 specimens contained a fentanyl-related compound with no detectable fentanyl. Two of these specimens contained only 4-ANPP. Of the 163 specimens in which fentanyl or a fentanyl-related compound was detected 31 (19%) had no other analytes detected. Using the method's LOD of 1 pg/mg as a cutoff, the detection rate for fentanyl was 14.7%. Increasing the cutoff concentration to 5 and 20 pg/mg decreased the detection rate to 10.2% and 7.1%, respectively. Most commercial testing laboratories utilize cutoffs between 20-100 pg/mg. For the 98 specimens with fentanyl concentrations in the quantifiable range (5-2000 pg/mg), the maximum, mean, and median concentrations were 1,946, 223, and 55 pg/mg, respectively. 7 specimens had concentrations greater than the ULOL with an estimated maximum fentanyl concentration of 9,246 pg/mg. 44 specimens contained detectable norfentanyl. The norfentanyl:fentanyl ratios ranged from 0.02 to 0.46 with a mean of 0.09.

Discussion: The detection rate for fentanyl in this COMDT population was third only to methamphetamine and hydrocodone. The fentanyl detection rate in this population was much higher than that of other opioids in workplace drug testing for the same time period. In this COMDT population fentanyl detection rates were 14.7% and 10.3% using cutoff concentrations

of 1 and 5 pg/mg, respectively. Fentanyl was detectable in hair from 1 pg/mg to an estimated 9,246 pg/mg. The fentanyl concentrations and norfentanyl:fentanyl ratios presented here may help inform cutoff decisions for programs adding fentanyl to their testing panels.

Disclaimer

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P-039

Using the New Stellar Mass Spectrometer to Confirm and Quantify 31 Drugs of Abuse, Including THC, in Oral Fluid

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Abstract

Introduction: As the clinical and forensic communities increasingly adopt oral fluid testing for its ease of collection and on-site evaluations, the need to detect a broad range of analytes with sufficient sensitivity becomes paramount. Meeting the LOQ targets outlined by SAMHSA demands both robust extraction methods and high-performance analytical instrumentation. This workflow—capable of extracting THC alongside other drugs of abuse—and the Stellar mass spectrometer combine comprehensive sample preparation with full scan MS^2 data, enhancing sensitivity, selectivity, and accuracy for detecting and quantifying drugs of abuse in oral fluid.

Objectives: To develop an LCMS method for quantitative analysis of 31 drugs of abuse in oral fluid with a complete sample preparation workflow that satisfies cutoff levels presented by the National Safety Council.

Methods: Eight calibration levels (ranging from 0.25 to 1000 ng/mL) were made by spiking stock solution of the 31 target analytes (including opioids, stimulants, cocaine, and THC) into human oral fluid. A customer's toxicology lab provided 10 case samples for this study. Calibrators and case study samples were diluted with a preserving buffer and spiked with their corresponding internal standard. 500 µL of each sample were then extracted using DPX INTip™ SCX/WAX SPE tips. Drug analytes were separated on a Thermo Scientific™ Accucore™ Biphenyl column connected to a Thermo Scientific™ Vanquish™ Horizon UHPLC system. Data was acquired on the Thermo Scientific™ Stellar™ mass spectrometer using targeted-MS2 (tMS2) mode with an inclusion list for the 31 target drugs. Optimized HCD or CID fragmentation was used to provide unique and high-intensity fragments. Thermo Scientific™ TraceFinder™ 5.2 software was used for data acquisition and processing.

Results: Chromatography exhibited ample separation for isomers including codeine / hydrocodone and morphine / hydromorphone. The experimental method addressed some of the issues commonly associated with THC, including absorbing to sample preparation consumables and its suppression by oral fluid collection device buffers. Limits of quantitation (LOQ) were determined for the 31 drugs. LOQ was defined as the back-calculated concentration where % difference and % RSD were less than 20%. All 31 drugs were confirmed with retention times and MS2 ion ratios and achieved as low as or lower LOQs than SAMHSA cutoffs. The ability to use HCD or CID fragmentation allowed for optimizing each compound based on the most favorable energies and fragments. Case study samples were analyzed, and statistical analysis was performed.

Discussion: This fast, quantitative Stellar mass spectrometry method was used to detect and confirm 31 drugs of abuse in oral fluid.

P-040

Determination of Nitazenes in Postmortem Casework and their Prevalence in the State of Maryland

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Abstract

Introduction: The nitazenes are the latest of several classes of novel psychoactive substances (NPS) to predominate the illicit drug market. Following the class-wide scheduling of fentanyl analogs in 2018, forensic toxicology laboratories in the United States began to see a proliferation of these novel synthetic opioids in casework initially stemming from the emergence of isotonitazene in 2019. Nitazenes are structurally distinct from fentanyl but share similar pharmacologic and toxicologic activity as mu-opioid receptor agonists with varying degrees of potency. Previous reports have shown that nitazenes are typically found at relatively low concentrations in casework (<10 µg/L), which suggests that these opioids may go undetected using traditional analytical workflows that include full scan gas chromatography-mass spectrometry with library matching. The prospect of new and emerging nitazenes in overdose fatalities has therefore prompted the development of a rapid, sensitive, and robust screening method utilizing ultra performance liquid chromatography-triple quadrupole mass spectrometry (UPLC-MS/MS) for postmortem casework encountered in the State of Maryland.

Objectives: This presentation describes an investigation initiated to better understand and characterize the prevalence and toxicological impact of nitazenes on postmortem casework throughout the State of Maryland. We additionally present a targeted multiple reaction monitoring method by reversed-phase UPLC-MS/MS for the qualitative determination of 11 nitazenes, of which include the recently emerging "*N*-desethyl" and "*N*-pyrrolidino" compounds along with two unique metabolites: 4'-hydroxy nitazene and 5-amino isotonitazene. The method was applied to postmortem casework received between 2024-2025. These findings will also be correlated to corresponding observations and additional toxicological findings in casework.

Methods: Target nitazenes were isolated from 1 mL of specimen utilizing a mixed-mode cationic exchange solid phase extraction protocol developed and validated for postmortem blood/fluids, urine, and solid tissue homogenate. Separation and mass spectral detection were achieved using a Waters Acquity UPLC coupled to a Waters Acquity Triple Quadrupole Detector. The UPLC-MS/MS was operated using electrospray ionization with positive polarity. Data was collected using multiple reaction monitoring with a minimum of two transition ions for each analyte and one transition ion for each internal standard. Positive identification was achieved using a known retention time and the detection of all appropriate transitions at the accepted ion ratio allowances. The method was validated in accordance with ANSI/ASB Standard 036.

Results: Four hundred and thirty* postmortem cases submitted to the State of Maryland Office of the Chief Medical Examiner between 2024-2025 were analyzed using this method, with 3% of cases positive for one or more nitazenes. The most prevalent nitazenes detected were metonitazene (62% of positive cases), *N*-desethyl protonitazene (31% of positive cases), and *N*-pyrrolidino protonitazene (31% of positive cases). Estimated concentrations ranged from 0.01-123 µg/L in blood/fluids and 0.07-3.77 µg/L in urine for all nitazenes and their metabolites positively identified. All positive cases had co-detected drugs. The most frequently co-detected drug was fentanyl (85% of cases), followed

by quinine (77% of cases), despropionyl fentanyl (69% of cases), cocaine/benzoylecgonine (62% of cases), and para-fluorofentanyl (54% of cases). The cause of death for all cases analyzed in this series was determined to be mixed drug intoxications with and without complicating disease states. (*The final presentation will include updated information as additional case data are collected.)

Discussion: The data collected during this investigation provides a unique insight into the toxicological impact of nitazenes in postmortem casework throughout the State of Maryland. The analytical method has additionally proven to be invaluable for postmortem casework where scene evidence, autopsy findings, and case history indicate possible drug use, but initial toxicology results are negative. However, with all positive cases in this series exhibiting a history of polypharmacy, this suggests the potential for the unknowing consumption of a more potent opioid than the user intended and highlights a low, but consistent presence of nitazene-positive cases that have continued to evolve over time in the illicit drug supply. More cases are expected to be positive through the continued use of this method along with continual additions of emerging nitazenes. Given the ever-changing landscape of NPS, the potency and increasing prevalence of these synthetic opioids raise significant concerns and underscore the need for ongoing surveillance, advancements in analytical techniques, and targeted public health interventions to mitigate their impact on mortality trends.

P-041

Using the New Stellar Mass Spectrometer and Automated Extraction for Drugs of Abuse Testing in Whole Blood

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Abstract

Introduction: As workloads increase in forensic laboratories, efficiency and robustness is of paramount importance. Creating faster methods for targeted panels can help improve productivity as well as implementing automation in the extraction process. Meeting the LOQ targets outlined by ANSI/ASB requires both robust extraction methods and high-performance analytical instrumentation with the sensitivity, selectivity, and accuracy for detecting and quantifying drugs of abuse in whole blood. This workflow on the Stellar mass spectrometer combines increased efficiency in the sample preparation with robustness in full scan MS^n data.

Objectives: To develop a whole blood LC-MS assay with automation for quantitation of drugs of abuse that satisfy levels specified in ANSI/ASB Standards 119 and 120.

Methods: Eight calibration levels (ranging from 0.25 to 5000 ng/mL) were made by spiking a stock solution of 87 target analytes (including opioids, stimulants, cocaine, and THC-COOH) into human whole blood. 10 real samples were provided by a customer's toxicology lab for a case study. 100 µL of each sample were then extracted by a protein precipitation using DPX Low Porosity ToT technology on the Hamilton Star. Drug analytes were separated on a Thermo Scientific™ Accucore™ Biphenyl column using a 5-minute method connected to a Thermo Scientific™ Vanquish™ Horizon UHPLC system. Data was acquired on the Thermo Scientific™ Stellar™ mass spectrometer using targeted-MS2 (tMS2) mode. Optimized HCD or CID fragmentation was used to provide unique and high-intensity fragments. Thermo Scientific™ TraceFinder™ 5.2 software was used for data acquisition and processing.

Results: Chromatography exhibited ample separation for isomers including codeine / hydrocodone and morphine / hydromorphone. Limits of quantitation (LOQ) were determined for the 87 drugs. LOQ was defined as the back-calculated concentration where % difference and % RSD were less than 20%. All drugs were confirmed with retention times, MS2, or MS3 ion ratios.

Discussion: This fast, quantitative Stellar mass spectrometry method with automation was used to detect and confirm drugs of abuse in whole blood. LOQ's for each drug satisfied the cutoffs specified in ANSI/ASB Standards 119 and 120. The ability to use HCD or CID fragmentation allowed for optimizing each compound based on most favorable energies and fragments. Case study samples were analyzed, and statistical analysis was performed.

P-042

An Improved Buffer-Surfactant System for Acute Organophosphate Poisoning Detection by Testing Human Red Blood Cell Acetylcholinesterase Activity

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Abstract

Introduction: Despite their effectiveness as pesticides, many organophosphates are dangerous anti-cholinesterase agents. Acute organophosphate poisoning can cause serious adverse conditions that may rapidly deteriorate to severe respiratory failure or death. Therefore, organophosphates have historically been used as poisons for nefarious purposes. For decades, depressed human red blood cell acetylcholinesterase (RBC-AChE) activity has been recognized as a critical biomarker for organophosphate poisoning. The U.S. government has utilized a modified Michel ΔpH method for RBC-AChE activity testing since the 1950s. Although the method is robust, it has safety drawbacks. Sodium barbiturate (NaB), a primary component of the buffer, is a controlled substance and can cause sedation and respiratory suppression. The cytotoxic RBC lysing agent, saponin, is capable of forming aerosol irritating the respiratory system. Our project was focused on a safer alternative buffer-surfactant combination that produces similar testing results compared to the current NaB-saponin system with minimal change of experimental settings.

Objectives: The purpose of this presentation is to describe an improved buffer-surfactant system for acute organophosphate poisoning detection by testing human RBC-AChE activity.

Methods: The NaB-phosphate buffer for the current method has an effective buffer range between pH 7.0 and 9.4. At the time of its development, this buffer was one of few covering the important physiological pH range. Today, several less toxic chemicals could form the buffer duplicating such a pH range. By titrating NaB, Tricine, Tris, Bis-Tris, and Tris-HCl individually of various ion strength, we discovered that NaB, Tris, and Tricine shared a buffer range at 0.01 M. The selection of surfactants was primarily based on their hydrophilic-lipophilic balance (HLB) value to enable a surfactant to behave like a detergent rather than a solubilizing agent. The current method utilizes saponin at 100 ppm which is well below its critical micelle concentration. After extensive testing, we found that both Triton X-100 (TX) and the blend Tween 80/Span 60 (90/10) (T80) could meet the requirement. The concentrations of TX and T80 leading to the hemolysis levels comparable to saponin (0.0120% w/v in the current method) were later determined to be 0.0220% and 0.0225% w/v, respectively.

Results: Several buffer-surfactant combinations were tested. Five (NaB-TX, Tris-TX, Tricine-saponin, Tricine-TX, and Tricine-T80) were further examined to determine if resulting ΔpH values coincided with those obtained via current NaB-saponin system near the 17-minute mark, which is the reaction termination time in the current method. The U.S. government considers the alternative system acceptable if the testing result is within the range of ±0.05 of the ΔpH of the same sample generated with the current method and prefers minimal change in reaction time. Briefly, the assay consisted of 200 µL of RBCs suspended in 4.0 mL of buffer containing the surfactant. Hydrolysis immediately started once 400 µL of acetylcholine was added. The pH value was recorded over time up to 30 minutes, and the ΔpH were calculated and compared to the ΔpH generated with the current method. The same RBC sample was split for the five alternative and the current buffer-

surfactant systems. Testing was conducted in triplicates with each system. At the 20-minute reaction time mark, both the Tricine-TX and the Tris-TX systems consistently produced the acceptable testing results with the smallest reaction time change. However, preference was given to the Tricine-TX system because the significant temperature dependence of pH associated with Tris-containing buffers was noticed. Interestingly, the potential problem of complex formations between Tricine and divalent cations was not observed.

Conclusion/Discussion: Considering the overall safety, efficiency, and ease of operation, we recommend the Tricine-TX combination as a safer and improved buffer-surfactant system for the federal RBC-AChE activity testing with slightly modified reaction time.

Disclosure

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

P-043

Evaluation of Hydrogen Carrier Gas for the Quantitative Analysis of Volatile Compounds in Biological Fluids using Headspace Dual Column Gas Chromatography with Flame Ionization Detection

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Abstract

Introduction: The commonly prescribed analytical technique for blood alcohol analysis has been headspace dual column gas chromatography flame ionization detection (GC-FID) using helium as the carrier gas. Of recent, the global helium crisis has left forensic science laboratories scrambling to evaluate new carrier gas options for gas chromatography methods. Hydrogen is a potential alternative carrier gas for gas chromatography techniques and can be amenable to headspace GC-FID methods.

The Toxicology Section of the Virginia Department of Forensic Science (DFS) evaluates biological specimens for the presence of alcohol and other drugs in criminal matters including driving under the influence of alcohol/driving under the influence of drugs (DUI/DUID) and death investigations. On average, DFS Toxicology receives approximately 5,000 DUI/DUID, 5,100 postmortem, and 800 other cases per year. Blood alcohol analysis is performed on all cases received in the Toxicology Section and is arguably the most common analytical test requested in forensic toxicology laboratories across the country.

Objectives: The objectives of the project were: 1) develop an analytical method using hydrogen as the carrier gas for the quantitation of methanol, ethanol, isopropanol, and acetone; 2) validate the optimized analytical method to meet the requirements set forth in ANSI/ASB Standard 036, *Standard Practices for Method Validation in Forensic Toxicology (1st Edition)*; 3) compare the validated analytical method against the existing helium carrier gas methodology using authentic biological specimens.

Methods: Sample dilution of biological fluids (blank blood, antemortem blood, postmortem blood, and vitreous) was optimized by evaluating a 1/5, 1/10 and, 1/20 dilution of specimen in internal standard (n-propanol). An Agilent Technologies headspace dual column GC-FID using hydrogen was optimized for the quantitation of ethanol and other volatiles. The optimization of the headspace parameters included the evaluation of vial size, equilibration time, vial agitation, and temperature. Additionally, the GC parameters were optimized using Agilent Technologies DB-ALC1 and DB-ALC2 GC columns and hydrogen as the carrier gas. The inlet temperature, gas flow, column temperature, and runtime were optimized to establish a working range of 0.010% w/v – 0.500% w/v for ethanol and 0.010% w/v – 0.400% w/v for the other volatiles.

Results: The hydrogen carrier gas method using a 20 mL sample vial size is being validated to ANSI/ASB Standard 036, *Standard Practices for Method Validation in Forensic Toxicology*. The sample preparation uses 50 µL of biological fluid diluted with 450 µL of internal standard into a 20 mL headspace vial. Samples are incubated for 4.0 minutes at 70°C with vial agitation (Level 5). The GC was established with an inlet temperature of 110°C, a gas flow of 27.9 mL/min, and a column temperature maintained at 45°C.

The elution order for the target compounds on the DB-ALC1 column was methanol (1.144 minutes), ethanol (1.425 minutes), isopropanol (1.723 minutes), acetone (2.057 minutes), and n-propanol (2.296 minutes). The DB-ALC2 column had slightly different elution times and a different elution order to provide selectivity. Bias and precision were evaluated using pooled fortified matrices at 0.030% w/v, 0.16% w/v, and 0.35% w/v with predetermined acceptance criteria of ±6% for ethanol and ±10% for methanol, isopropanol, and acetone. Commonly encountered interferents, estimated limit of detection (0.01% w/v), and lower limit of quantitation (0.01% w/v) were established and is being validated.

Discussion: A method has been developed and is being validated for the quantitative analysis of volatile compounds in biological fluids. The method presents the utilization of hydrogen carrier gas to help laboratories cope with the dwindling nonrenewable helium supply. In addition to modifying the carrier gas, an extensive investigation into the sample preparation (e.g., dilution and incubation parameters) has been evaluated. This method provides an alternative approach for one of the most commonly evaluated analytes in forensic toxicology.

P-044

Launch of Resource Directory for Knowledge Transfer Between Scientific Instrument Users

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Abstract

Introduction: The Society of Forensic Toxicologists (SOFT) Applied Analytical Toxicology (AAT) and Toxicology Resource Committees (TRC) have been working to establish a Resource Directory to enhance collaborations between laboratories using similar analytical techniques and instrumentation. The creation of this directory has been a multiyear project that included an initial broadcast for interested participants followed by analytical technique/instrument specific questionnaires. The establishment of a repository is complex and multifaceted but has the potential to strengthen information sharing within the forensic toxicology community. Once established the AAT and TRC will manage the directory and facilitate working group meetings and communication between laboratories.

Objectives: The objective of this project is to promote interlaboratory communication and collaboration within the forensic toxicology community.

Methods: A question was added to the 2023 SOFT Laboratory Survey from the TRC to obtain contact information from participants interested in being part of the Resource Directory. From the list of interested participants, a second questionnaire was established to narrow down specific areas of interest for each respondent. Once established, instrument specific questionnaires were created to include information regarding what types of methods the instrumentation is being used for, sample preparation procedures, and instrument specific information. This information was filtered and sorted to establish a framework for the repository.

Results/Discussion: From the 2023 SOFT Laboratory Survey, a total of 90 interested individuals were identified. These individuals were directly contacted to obtain their interest in different analytical techniques and instrumentation, including a but not limited to, colorimetric tests, immunoassay, gas chromatography coupled to a variety of different detectors, liquid chromatography tandem mass spectrometry, and high-resolution mass spectrometry. Individuals had the option to select all categories of interest. From there, individual questionnaires were created beginning with high resolution mass spectrometry.

A total of 39 individuals were interested in being a part of the high-resolution mass spectrometry directory and a total of 44 responses were received. Once the information was obtained, the AAT worked to parse through the data to establish a draft directory. A second survey was then created and disseminated to 69 individuals interested in being involved with the tandem mass spectrometry directory. The results from the survey are being organized to establish a draft directory for tandem mass spectrometry.

The resource directories will serve as a centralized repository for individuals to discover, access, and share information regarding analytical methods and instrumentation within the forensic toxicology community. As the directories begin to form and expand, additional individuals are welcome to express interest in joining the project and becoming a part of the growing directory community.

P-045

Rapid EtG/EtS Analysis in Normal and Disease State Urine by LC-MS/MS

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Abstract

Introduction: Ethyl Glucuronide (EtG) and Ethyl Sulfate (EtS) are established biomarkers for alcohol consumption. The analysis of these compounds poses challenges due to their polar nature, making them challenging to retain by reversed phase chromatography. Additionally, there are isobaric matrix interferences in urine that require full chromatographic resolution from the analytes to obtain accurate data. Disease state samples provide an additional challenge due to the potential of unique interferences. In this work, a rapid LC method was developed that demonstrated excellent resolution between the matrix interferences and the analytes of interest.

Objectives: This study aimed to develop a robust LC-MS/MS method capable of retaining both EtG and EtS while resolving urinary matrix interferences in both normal and disease state patient samples.

Methods: Calibrators were prepared in synthetic urine fortified with EtG and EtS from 50-1,000 ng/mL. Quality control (QC) samples were prepared using six lots of human urine, including three from single-donor disease state patients (kidney disease, liver disease, diabetes). Samples were diluted 20-fold in water fortified with 0.1% formic acid. The samples were then vortexed and centrifuged prior to a 10 µL injection on a Force Biphenyl column (100 x 3 mm, 3 µm) at 30°C. The mobile phases used were water and methanol, both modified with 0.1% formic acid (v/v), and the chromatographic separation was achieved under gradient conditions starting at 100% aqueous. Precision and accuracy experiments were performed on a Shimadzu LCMS-8060 triple quadrupole using electrospray ionization in negative ion mode.

Results: The developed method successfully resolved matrix interferences from the analytes of interest. No significant matrix interferences were observed across the QC urine lots including those in disease state samples. Linearity was demonstrated for both analytes with $r^2 \geq 0.99$, and the method exhibited acceptable (+/-10%) intra- and inter-day precision and accuracy. The use of a fully porous Biphenyl column enabled adequate retention for these polar analytes to separate matrix interferences.

Discussion: A rapid and reliable LC-MS/MS method was developed for the quantitation of EtG and EtS in urine using a fully porous Biphenyl column. The method was verified across multiple lots of human urine and disease state samples, offering a robust and versatile solution for clinical and forensic applications. It also provides flexibility for analyzing a broad range of compounds, including drugs of abuse and novel psychoactive substances, reducing the need for column changes.

Conflict of Interest

2) Salary

P-046

Unmasking Isobutylene: A Rare Toxicity from a Widely Used Industrial Gas

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Abstract

Introduction: Isobutylene (isobutene, 2-methylpropene) is a highly flammable, colorless gas primarily used as a chemical intermediate in the petrochemical industry. It is a key monomer in butyl rubber, commonly found in pharmaceutical stoppers. As such, isobutylene may be identified as a contaminant in toxicological specimens collected in tubes with conventional rubber stoppers. Though isobutylene is produced and handled in large volumes, reports of human toxicity are rare. Inhalation of isobutylene at high concentrations may cause dizziness, headache, respiratory irritation, CNS depression, and loss of consciousness but clear thresholds and exposure data in humans are limited. Here we report a case of a 33-year-old male who was witnessed to collapse inside a tank car within 10-15 seconds after attempting to assist a colleague who had previously entered the tank car and lost consciousness. Rescue efforts found the individual unresponsive, face-down in several inches of standing water wearing a respirator full of water. Lifesaving measures were unsuccessful. The other individual was transported to the hospital and survived. Both individuals were washing out the tank car which was reported to previously contain isobutylene. The day prior, the gas was “burned off” and left to air out for 24 hours.

Objectives: This poster presents a suspected case of isobutylene toxicity with associated symptoms and discusses the diagnostic challenges involved in confirming exposure to such an uncommon compound.

Methods: All postmortem specimens were collected at autopsy at the Travis County Medical Examiner (TCME) in Austin, TX, including femoral and aortic blood, vitreous, brain, and lung tissue. Femoral blood was screened for volatiles utilizing headspace dual-column gas-chromatography with flame ionization detection (HS-GC-FID) and drugs of abuse by immunoassay (ELISA) for four drug classes (Barbiturate, Benzodiazepine, Opiate, THC). Qualitative drug screening on the femoral blood was performed by LC-QTOF-MS. Non-routine qualitative volatiles analysis was also performed via headspace GC-MS with an Agilent J&W GS-GasPro column in full scan data acquisition mode.

Results: Autopsy findings demonstrated a well-developed, obese male with cerebral and pulmonary edema. The right and left lungs weighed 780g and 720g, respectively. These nonspecific findings can be observed in cases of drowning and drug toxicity. Routine toxicology testing was presumptive positive for cannabinoid metabolite only. However, an unidentified peak eluting at the same retention time as methanol was observed in column one following volatiles analysis. Combined with the suspected exposure to isobutylene, non-routine volatiles analyses were conducted on the blood, brain and lung tissue. The presence of isobutylene was confirmed in all specimens above thresholds established from negative specimens collected in red-top tubes (n=10). Femoral blood was sent to a reference laboratory for further volatiles screening with no additional compounds detected.

Following a thorough death investigation, autopsy, and toxicology, the cause of death was certified as “drowning”, and the manner of death an accident. The “how injury occurred” section

of the death certificate included “drowned in a puddle of water inside a tank car following loss of consciousness resulting from the toxic effects of isobutylene.”

Conclusion/Discussion: This case was the first documented instance of death related to isobutylene exposure at the TCME. Although ultimately not the cause of death, the decedent rapidly lost consciousness upon exposure to isobutylene in a confined space. Information regarding the toxicity of this compound is scarce, and toxicological interpretation is complicated by the presence of isobutylene in many of the rubber stoppers used in blood collection tubes. It is important for laboratories to establish baseline thresholds in headspace samples to differentiate between isobutylene exposure and potential exogenous contamination from the rubber stoppers. Additionally, laboratories should be aware of the potential misidentification of isobutylene as methanol with single column HS-GC-FID methods.

Key Words

Isobutylene, volatiles analysis, postmortem toxicology

P-047

The Cheesehead Truth: A 5 Year Review of Wisconsin Impaired Driving Cases

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Abstract

Introduction: The Wisconsin State Laboratory of Hygiene reviewed data from operating while intoxicated (OWI) and motor vehicle death (MVD) cases submitted for alcohol and drug analysis from 2019 to 2023. The total number of cases involved in the data pool was 99,613 and included submissions from all 72 counties in Wisconsin.

Objectives: The objective of this project is to determine and evaluate trends in OWI and MVD samples between the years 2019-2023 from the state of Wisconsin.

Methods: A review of five years of OWI and MVD samples for the state of Wisconsin including alcohol and drug results, sex, age, time of driving, and severity of incident. All age and sex subject information were collected from driver's license data. Drugs investigated were buprenorphine, benzodiazepines, cocaine, fentanyl, methamphetamine, MDMA, opiates, and THC.

Results: The following is a summary of notable trends relating to sex, age, and time of incident. Over the 5 years, the number of submissions from males was 3 times the number of submissions from females. The dispersion of the blood alcohol concentration (BAC) results and the ages of males and females were consistent. Men and women also followed similar patterns in the type of drugs used in OWI cases. Sex did not have a significant impact on the percentage of cases that resulted in crashes, injuries, and fatalities. Men and women in the 50 to 59-year-old age group had the highest average BAC at 0.176g/100mL and 0.178g/100mL, respectively. The average BAC for all cases was 0.172 g/100mL. The 21 to 29-year-old age group had the most overall submissions, as well as cases that involved a crash or injury, while the 30 to 39-year-old age group had the most fatal crashes. The average BAC for crashes over the 5 years was 0.191 g/100mL; for crashes involving injury, 0.171 g/100mL; and for crashes involving fatalities, 0.134 g/100mL. Individuals aged 21 to 39-years-old were most common to use multiple drugs and drive. Those over 80 were found to not have more than 2 drugs in their blood at the time of their violation. The 30 to 39-year-old age group was the group with the most cocaine, methamphetamine, MDMA, fentanyl, buprenorphine, benzodiazepines, and opiates usage. The age group with the most THC usage was the 21 to 29-year-olds. THC is the most common drug for every age group except for 80+. Over the 5 years, some of the most common drug combinations were THC and methamphetamine, THC and cocaine, and fentanyl and benzodiazepines. As age rises, the time of a violation moves away from being between 12 AM and 6 AM and moves towards violations between 6 PM to 12 AM. The most common time of day for crashes, regardless of severity, occurred between 6 PM and 12 AM. The most common time for any OWI and MVD cases was between 12 AM and 6 AM. Fatal crashes were least likely to occur between 12 AM to 6 AM.

Discussion: Through the review of this data, we can see that there is still a large problem in Wisconsin with impaired driving, especially for those under the age of 40. These trends are only a small portion of the data available for OWI and MVD cases. In the future we look to expand this project to encompass more factors and years of data to better understand OWI and MVD trends over the years. We also want to look towards other crime laboratories to better understand these trends.

P-048

Cannabinoid Profiling Across Toxicology Samples in Adolescents and Young Adults by Route of Administration and in Relation to Depression Symptoms

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Abstract

Introduction: Cannabis use is common, with recreational cannabis legalized in 24 US states. Concerningly, 36% of 12th graders and 42% of young adults (ages 19-30) report past year cannabis use. With an expanding retail market, diversity in cannabis products contributes to difficulty in accurately assessing the impact of cannabis use in vulnerable populations such as emerging adults. Toxicological cannabinoid concentrations may clarify cannabis relationships despite variability in cannabinoid products and use patterns. This study aims to consider self-reported cannabis use days with toxicological results across matrices to assess alignment across matrices and route of administration, with a focus on THCCOOH—a long-term THC metabolite. Finally, if cannabinoid concentrations offer a more consistent picture of cannabis use than self-report, different matrices may more sensitively predict clinical relationships. Thus, THCCOOH concentration across matrices are considered as a predictor of depression symptoms, as depression is established as being linked to cannabis use.

Objectives: 1) Assess THCCOOH concurrence between self-report and across matrices (oral fluid, plasma, urine, and hair); 2) examine whether route of administration (i.e., flower and vaped concentrate) impacts THCCOOH concentration across matrices; and 3) investigate predictive utility of THCCOOH concentration across matrices on depression symptoms.

Methods: Cannabis using (n=70) and non-using (n=24) adolescents and young adults (64% female; ages 18-21) were asked to contribute oral fluid, blood, urine, and hair for toxicological testing and self-reported past-90 days of cannabis use, including route of administration. Plasma, urine, and hair THCCOOH were quantified using validated LC-MS/MS assays (for plasma and urine) and GC-MS/MS (for hair). Positive and negative toxicological results by matrix are presented, with sensitivity and specificity calculated. Correlations between THCCOOH concentration across matrices and self-report use were run. Analysis of variance models (ANOVAs) tested whether product type (smoked flower v. vaped concentrate) influenced cannabis use patterns, use to avoid withdrawal, or THCCOOH concentration across matrices. Regressions assessed cannabis metrics predicting depression symptoms, controlling for biological sex.

Results: All matrices demonstrated excellent specificity (100%), with largely adequate sensitivity (63-74%) except for oral fluid (12%). Self-report and toxicological metrics were significantly correlated (r's=.41-.97), except for self-reported use to avoid withdrawal. THCCOOH concentrations across matrices did not differ by route of administration group; groups also did not differ by self-reported use days or avoiding withdrawal symptoms (*p*'s=.16-.66). Only plasma THCCOOH concentrations predicted depression symptoms (standardized beta=0.47, $p<.001$), indicating a moderate-to-large effect size.

Discussion: Taken together, THCCOOH concentrations in toxicological matrices and self-reported cannabis use offer concurrent information in adolescents and young adults who regularly use cannabis. THCCOOH concentrations did not differ significantly by typical and preferred route of cannabis administration (smoked flower or vaped concentrate), nor did other cannabis outcomes (self-reported use or using to avoid withdrawal symptoms). Plasma THCCOOH concentration uniquely predicted self-reported depression symptoms. This may indicate that toxicological cannabinoid concentrations are beneficial for predicting clinical outcomes. Given the complexity of measuring cannabis use due to the plethora of available products and rise of new popular cannabinoids, use of toxicological results may offer new insights into clinical outcomes in those who frequently use cannabis.

P-049

Enhanced Sample Preparation in Urine Drug Testing: A Comparative Study of Enzymatic Hydrolysis Methods for Pain Management Drugs with Focus on Morphine and Its Glucuronide Metabolites

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Abstract

Introduction: Enzymatic hydrolysis is a cornerstone step in the preparation of urine specimens for opioid testing. Many opioids, including morphine, are excreted primarily in their glucuronidated forms, most notably as morphine-3-glucuronide (M3G) and morphine-6-glucuronide (M6G), which must be effectively cleaved to allow accurate quantification of free morphine. Incomplete or inefficient hydrolysis may lead to false-negative results, underreporting of drug use, or misinterpretation in forensic, clinical, and pain management settings.

This study presents a comprehensive evaluation of three novel enzymatic hydrolysis formulations (E1, E2, and E3) developed by Kura Biotech, designed for efficient hydrolysis at room temperature, with the aim of improving accuracy, operational simplicity, and reproducibility in routine urine drug testing.

The enzymatic performance of these new homologous formulations (E1, E2, and E3) was benchmarked against two commercially available standards: Kura B-one®, a widely used β-glucuronidase preparation for room temperature use, and Kura BG Turbo®, an established high-temperature enzymatic solution. Special focus was placed on the liberation of total Morphine which includes free morphine and the two major glucuronide conjugates of morphine (M3G and M6G).

Objectives:

- To assess and compare the hydrolysis efficiency of E1, E2, E3, and commercial enzymes (Kura B-one® and Kura BG Turbo®) for the conversion of M3G and M6G into free morphine.
- To develop a large-panel LC-MS/MS urine drug testing method for pain management drugs and metabolites, supported by enzymatic hydrolysis and dilute-and-shoot sample preparation.
- To evaluate the impact of hydrolysis conditions (temperature, incubation time) on the release of parent morphine from conjugates.
- To establish the clinical and forensic utility of improved hydrolysis agents in monitoring compliance, drug metabolism, and potential opioid misuse.

Methods:

Sample Preparation: Urine samples were prepared using a dilute-and-shoot strategy with enzymatic hydrolysis performed under the following conditions:

- Mix in a 1.8 mL Eppendorf tube:
    - 50 µL urine (each of blank, double blank, calibrators, QCs and Patient samples)
    - 40 µL internal standard

    - 20 µL enzyme mix (E1, E2, E3, Kura B-one®, or Kura BG Turbo®)
    - 455 µL LC-MS grade water
    - Vortex to mix
- Incubation:
    - Room temp for 15 min (E1, E2, E3, Kura B-one®)
    - 55°C for 30 min (Kura BG Turbo®)
- Add 335 µL of sample diluent (20% 0.1% FA methanol in 0.1% FA water)
- Vortex and centrifuge at 12,700 RPM for 15 min
- Transfer 700 µL supernatant to 1 mL auto-sampler vial
- Inject 10 µL into LC-MS/MS system

Instrumentation and Chromatography: LC-MS/MS analysis was conducted using an AB Sciex 4000 Q-Trap with electrospray ionization, coupled to a Shimadzu LC system. Separation was achieved on a Phenomenex 2.6 µm Phenyl-Hexyl (50 × 4.6 mm) column.

- **Mobile Phase A:** 10 mM ammonium formate with 0.1% formic acid in water
- **Mobile Phase B:** 0.1% formic acid in methanol
- **Internal Standards:** Matched deuterated IS for each analyte
- **Calibration/QC:** Prepared in drug-free urine using diluted certified reference standards

Test Panel: The developed panel included 56 pain management drugs and their metabolites, covering both free and glucuronidated forms M3G and M6G were specifically monitored in every analytical run to benchmark enzymatic performance.

Results:

- E1 demonstrated superior hydrolysis efficiency, achieving up to 98.5% conversion of M3G and M6G to free morphine at room temperature within just 15 minutes.
- E2 and E3 also showed strong performance but with slightly lower conversion rates (96–98%) under the same conditions.
- Kura B-one® produced moderate hydrolysis efficiency (94–97%) but exhibited greater variability between runs.
- Kura BG Turbo®, though effective (92–97% conversion), required elevated temperature (55°C) and a longer incubation period (30 min), potentially increasing workflow complexity and energy use.
- All E1, E2 and E3 homologous formulations showed minimal analyte degradation, excellent reproducibility, and lower matrix interference in LC-MS/MS analysis.
- M6G hydrolysis was historically more challenging, but E1 notably outperformed all other enzymes in consistently releasing free morphine from M6G.

Conclusion: This study highlights the importance of enzyme choice in urine drug testing. The new E1 enzyme from Kura Biotech is efficient, reliable, and easy to use—working well at room temperature with less time and no heating. It enables fast and complete hydrolysis of morphine glucuronides, improving test accuracy and sensitivity. E1 is well-suited for routine LC-MS/MS use in clinical, compliance, and forensic settings, helping support confident decision-making.

Conflict of Interest

Grant/Research Support from Kura biotech.

P-050

Blood Alcohol and Inhalants of Abuse Analysis Using Gas Chromatography Optimized with Computer Software.

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Abstract

Introduction: Screening for volatile inhalants of abuse, as well as analyzing blood alcohol content, is commonly performed in forensic toxicology laboratories using headspace gas chromatography with flame ionization detection (HS-GC-FID). The analyses are performed using dual columns with specialized stationary phases that optimally separate these volatile compounds. While separation profiles of standard blood alcohol screening compounds are usually well characterized by column manufacturers on these application specific columns, elution profiles of inhalants may not be as readily available. In addition, providing example chromatograms with static run conditions may not suit laboratories, who want to experiment with faster run times, column dimensions, carrier gases, etc.

These issues can be solved by using computer modeling software to predict retention times of compounds of interest on a various stationary phases. In addition to the ability of the web-based software to help select a column and provide an optimized separation of compounds of interest on a specific stationary phase, the software can also be used to make changes to analytical conditions and observe the effect on elution, making it a valuable tool for method development and optimization.

Objectives: The intent of this project is to present retention time models for inhalants of abuse and blood alcohol analytes of interest on four unique stationary phases, using web-based modeling software and verify accuracy of the models against actual analyses. This will allow for optimized separations with faster separations in addition to the evaluation of different carrier gas types.

Methods: To build a database for computer modeling of chromatographic separations, the following fused silica capillary columns were installed into an Agilent 7890A GC with a 5975C MSD: Rtx-BAC1, Rtx-BAC2, Rtx-BAC Plus 1 and the Rtx-BAC Plus 2. More than 70 volatile inhalants of abuse, including solvents, refrigerants, nitrites (aka “poppers”) and their metabolites were analyzed on each column using three different temperature programmed run conditions. Two of the analyses were used to create a retention model based on thermodynamic indices of analytes and the third analysis was used to verify accuracy against the theoretical model. Once the models were finalized a web-based modeler, was used to optimize separations on each column, decrease analysis times, translate to different column dimensions or carrier gases, and make user input adjustments to parameters such as carrier gas flow rate and oven ramp rates.

Results: Confirmation runs were in agreement with the theoretical modeled analysis, demonstrating acceptable accuracy of the retention time models using all four columns. Selection of various compounds of interest in the software successfully generated optimized separations on each column, allowing the user to choose the column or column set that best fits their needs. The ability to optimized the method using different carrier gases, temperatures, column flows, different column dimensions and film thicknesses was clearly demonstrated.

Discussion: Computer modeling of retention times in GC is a valuable tool to aid in column phase selection and method development/optimization. The use of this software greatly reduces the time required for manual method development, since input changes update the results instantaneously. With libraries of 70+ volatile compounds on four different phases, users can select or input compounds of interest and then calculate elution profiles on each column. The software will present the number of coelutions on each column, allowing the user to select the most appropriate column for their analysis.

P-051

Differentiation of Fentanyl and its Isomeric Analogs *Ortho/meta/para*-methylacetylfentanyls for Federal Workplace Drug Testing by LC-MS/MS

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Abstract

Introduction: Fentanyl and its analogs represent a group of potent synthetic opioids that pose an alarming threat to public health in the United States and are often difficult to differentiate due to similar retention times and shared major fragmentation pathways. It is particularly challenging with fentanyl and its emerging isomeric methyl-analogs. As the only Department of Defense laboratory certified for federal civilian drug testing, we were tasked by the National Laboratory Certification Program (NLCP) to 'demonstrate the ability to prevent false fentanyl positive reports (in the presence of *ortho/meta/para*-methylacetylfentanyls), although baseline separation is not required.' As a result, our impression was that differentiation of fentanyl and *ortho/meta/para*-methylacetylfentanyls could be challenging if solely relying on chromatographic separation.

Objectives: To describe a practical and validated fentanyl LC-MS/MS confirmatory analytical method using unique ion transitions as critical chemical markers for differentiation of fentanyl and *ortho/meta/para*-methylacetylfentanyls for federal workplace drug testing.

Methods: Briefly, a solid phase extraction was performed with the urine specimen (500 µL) containing the internal standards (fentanyl-d5 and norfentanyl-d5, 1 ng/mL for each). The sample was eluted with 1 mL of 95:5:2 (v:v:v) EtOAc:MeOH:NH_4OH, dried under nitrogen, and re-constituted with 50 µL of 90:10 LC Mobile Phase A:Mobile Phase B. Confirmation was performed on an Agilent 1290 LC with a phenylhexyl column coupled to an Agilent 6470 triple quadrupole MS/MS (for a summary of LC-MS/MS settings, see Table 1). Fentanyl linear quantification range is 0.3-100 ng/mL (without presence of *meta*- or *para*-methylacetylfentanyl). Method was validated in accordance with the NLCP requirements.

Results and Discussion: Initially, *ortho/meta/para*-methylacetylfentanyls all partially coeluted with fentanyl. Additionally, they shared the two dominant and commonly used fentanyl ion transitions (m/z 337.2$(M+H)^+$ to 188.1 and 105.1) and their ion ratios for each transition were almost identical. Therefore, a failed ion ratio may not be a reliable indicator for the presence of *ortho/meta/para*-methylacetylfentanyls even with narrowed ion ratio acceptance ranges. We examined numerous LC parameter adjustments and different types of LC columns. Currently, baseline separation between fentanyl and *ortho*-methylacetylfentanyl was achieved with a phenylhexyl column, while *meta*- and *para*-methylacetylfentanyls each still partially co-eluted with fentanyl. This limitation led us to focus on the fragments preserving the subtle structural differences between fentanyl and *ortho/meta/para*-methylacetylfentanyls. Under ESI MS/MS condition, fentanyl is reported to undergo a 4-centered rearrangement, resulting in loss of the propionyl group to form m/z 281.2. We speculated that *ortho/meta/para*-methylacetylfentanyls would share the same transformation to yield m/z 295.2 with one more methyl group on the aniline benzene ring. Similarly, we proposed a second set of potentially unique fragments m/z 244.2 and 230.2 for fentanyl and *ortho/meta/para*-methylacetylfentanyls, respectively. Further validation confirmed the uniqueness and feasibility of the proposed ion transitions with no

'transition crosstalk' observed when fentanyl (as low as 0.3 ng/mL) mixed with one or more *ortho*/*meta*/*para*-methylacetylfentanyls (up to 100 ng/mL for each). Subsequently, we incorporated the transitions m/z 337.2 to 281.2, 295.2, 244.2, and 230.2 into the MS/MS data acquisition method with the minimum abundance threshold set to 200 counts for each transition to serve as critical and practical chemical markers to determine the presence of fentanyl and *ortho*/*meta*/*para*-methylacetylfentanyls. In addition, we were able to separate *ortho*-methylacetylfentanyl from *meta*- and *para*-methylacetylfentanyls under the LC condition.

Conclusion: To our best knowledge, our laboratory is the first to report utilization of unique ion transitions as critical chemical markers for differentiation of fentanyl and *ortho*/*meta*/*para*-methylacetylfentanyls for federal workplace drug testing. Although currently we are unable to provide fentanyl quantitation results with *meta*- or *para*-methylacetylfentanyls present, we are able to establish a reliable safeguard to prevent false fentanyl reports when sample contains *ortho*/*meta*/*para*-methylacetylfentanyls.

Table 1. Summary of LC-MS/MS Settings

LC Settings				
Agilent Poroshell 120 Phenylhexyl column (4.6x50 mm, 2.7µm)				
Injection Volume: 2 µL			Total Run Time: 12 min	
Mobile Phase A: 0.1% formic acid in water			Mobile Phase B: 0.1% formic acid in 50:50 acetonitrile:methanol	
LC Gradient and Timetable				
	Time (min)	Mobile Phase A (%)	Mobile Phase B (%)	Flow Rate (mL/min)
1	0.00	70.00	30.00	0.5
2	2.00	70.00	30.00	
3	10.00	40.00	60.00	
4	10.01	10.00	90.00	
5	11.00	10.00	90.00	
6	11.01	70.00	30.00	
MS/MS Settings				
Ion Source: ESI		Scan Type: MRM	Polarity: Positive	Data Acquisition: 0.2-10 min
MRM Transitions				
Compound	Typical Retention Time (min)	Precursor Ion	Product Ion	Comments
fentanyl	8.73	337.2	188.1	
			105.1	
			281.2	Chemical markers unique to fentanyl
			244.2	
Methylacetyl fentanyls	*ortho*- 8.12 *meta*- 8.55 *para*- 8.76	337.2	188.1	
			105.1	
			295.2	Chemical markers unique to methylacetylfentanyls
			230.2	
fentanyl-d5	8.70	342.3	188.1	
			105.1	

Disclosure

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

Conflict of Interest

No conflict of interest.

P-052

Determining the Accuracy of the N-Propanol Peak Area Statement in Postmortem Blood Volatiles Casework

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Abstract

Introduction: N-propanol is a widely used internal standard for blood volatiles analysis due to its similar structure and properties to ethanol and other volatiles of interest in forensics. However, it is known to be produced endogenously in postmortem samples. This poses a problem as it can affect the quantitation of ethanol and other volatiles when endogenous n-propanol is present in the sample.

The Oregon State Police toxicology section currently utilizes a 0.05% (v/v) internal standard for both antemortem and postmortem blood volatiles analysis. For postmortem analysis, an internal standard calculation worksheet is utilized to "flag" samples that may contain endogenous n-propanol. To do this, calculations are performed that take the mean peak area for all the samples in the batch and will flag the sample as elevated or high if the peak area is greater than 2 or 3 standard deviations from the mean. If a sample has duplicate aliquots that are flagged elevated or high for n-propanol with a positive ethanol result, a reporting statement is added stating: "analysis of Exhibit X indicated the presence of endogenous n-propanol in the sample. Due to this contamination, the reported ethanol result may be artificially low."

Objectives: Through the testing of postmortem specimens at the Oregon State Police crime lab, the effects of this interference were studied. The goal of this was to determine the accuracy of the current reporting statement utilized by the Oregon State Police crime lab when a sample is determined to have endogenous n-propanol. Over 50 postmortem blood specimens previously tested for volatiles for the State of Oregon Medical Examiner's office underwent a series of tests to determine the significance of endogenous n-propanol interference in the quantitated value of ethanol for those samples.

Methods: Selected samples for testing included 48 regular samples and 27 samples with "elevated" n-propanol results from original testing. 30 samples that tested negative for ethanol were also examined. Samples were prepared in a 1:6 dilution of 0.05% (v/v) n-propanol, and 0.02% (v/v) *tert*-butanol in water. Results using the currently established method with n-propanol as the internal standard were compared with a proposed method using *tert*-butanol as the internal standard. This analysis was performed to determine the impact of endogenous n-propanol to the quantitated ethanol value that would be released to the medical examiner's office in the state of Oregon using the current method.

Results: Among the 27 samples compared, the maximum relative difference between testing with *tert*-butanol and n-propanol as an internal standard for elevated n-propanol samples was 24.00%, with a mean relative difference of 7.96% across a range of ethanol concentrations [0.007-0.311 (g/dL)]. While additional testing may be necessary to further understand the scope of this topic, results at this point suggest that the quantitated ethanol result would not be significantly impacted by the presence of endogenous n-propanol that is typically seen in postmortem specimens.

Discussion: Volatiles testing is routine for toxicology labs throughout the world. It is important to provide accurate results to requesting agencies. Although endogenous n-propanol has the potential to cause an artificially low result for quantitated ethanol when analyzed using n-propanol as an internal standard, the quantity of n-propanol typically produced postmortem may be negligible in practical applications.

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P-053

Validated Oral Fluid and Urine Toxicology Screening RUO Workflows With DART-MSMS

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Abstract

Introduction: DART-MSMS, Direct Analysis in Real Time, was introduced in the recent past as a technology capable of providing analysis time in seconds, MS level data, and semi to fully quantitative assays. Currently, most toxicology screens use immunoassay which suffer from a range of well-known shortcomings; false positive, false negatives, cross-reactivity, limited coverage across the toxicology target range, and long development time for new assays.

Objectives: Develop and validate a quick, semi-quantitative screening toxicology, research use only (RUO), assay for oral fluids and urine. Demonstrate a workflow capable of replacing immunoassay (IA) as a screening technique. Confirm the results of this screening assay against a traditional LC-MS platform.

Methods: Multi-drugs kits were provided by Pinpoint Testing, LLC. Kits contain regression curves and quality control samples for all drugs, in oral fluid matrix. The experimental work was performed on a Bruker DART-TQ+ platform. All drugs were tuned and optimized. A sample preparation method was optimized and transferred to a robotic platform (Opentrons), on which samples were extracted. Extracts were spotted on a Bruker HTS96 screen using a Rainin MicroPro20 system. Analysis was performed with DART, in pulse mode, on a Bruker TQ$^+$ MS detector. The screened sample were subsequently confirmed on a Bruker LC-TQ$^+$ MS.

The DART screening method is due to undergo validation according to ANSI/ASB Standard 036.

Results: MS/MS transitions and collision energies were identified for all target compounds followed by optimization of scan time (20 ms), cone temperature (300°C), and collision cell pressure (1.5 mTorr). Optimized DART temperature ranged from 250-350°C, depending on drug target. Grid voltages were optimized to 50 V for all drugs. Concentration range varies depending on specific drug cutoff. Data were processed via standard MS quantitation software, using internal standards.

Initial data shows the validity of the technique for toxicology screening. Six standards were used to generate a regression curve; the resulting R2 was typically 0.985 or better. Three sets of QCs were used, one below the cutoff, one at cutoff and one above cutoff. Blank QCs were also run and were always negative.

Cross-validation of the samples demonstrated good correlation with LC-MS data, in that all samples that screened positive by DART were confirmed positive and all that screened negative were confirmed negative. This indicates that this rapid, chromatography-free workflow is sufficient in determining the status of the sample at or below the common cutoff values with improved accuracy compared to IA based screening approaches. There was no evidence of significant false positives or false negatives.

Validation of the screening assay as per ANSI/ASB Standard 036 is ongoing and results will be reported.

Discussion: This work indicates that the rapid, chromatography-free workflow is sufficient in determining the status of the sample at or below the common cutoff values with improved accuracy compared to IA based screening approaches. There was no evidence of significant false positives or false negatives. Validation of the screening assay as per ANSI/ASB Standard 036 is ongoing and results will be reported.

Conflict of Interest

The authors are employed by Bruker Corp.

P-054

Some Old Drugs Never Go Out of Style: Quantitation of Phencyclidine (PCP) by Gas Chromatography with Mass Spectrometry (GC-MS) in Postmortem Toxicology Cases (2023-2025)

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Abstract

Phencyclidine, or PCP, is a dissociative anesthetic drug of the arylcyclohexylamine class that has both history in medicine and recreational use. It was originally developed in the 1950s as an anesthetic but was later discontinued in humans due to severe adverse effects, including agitation, hallucinations, and delirium. It first appeared as an illicit recreational drug in the 1960s. PCP is an antagonist of the N-methyl-D-aspartate (NMDA) receptors, but it has other pharmacological mechanisms of action including effects within the cholinergic, adrenergic, and dopaminergic systems. As an illicit drug, PCP can be snorted, orally ingested, and injected, but it is primarily smoked either on its own or after mixing with other substances such as cannabis, tobacco, or cocaine. Adverse effects are dose-dependent, with higher dosages eliciting more significant physiological and psychological effects, which may include hypertension, tremors, euphoria, anxiety, dissociation, paranoia, delusions, arrhythmia, seizures, catatonia, and death.

Postmortem blood specimens were collected in gray top tubes containing sodium fluoride as a preservative and submitted to the laboratory for toxicological analysis. Toxicological testing included volatiles analysis by headspace gas chromatography with flame ionization detection (GC-FID) and a broad-spectrum screen targeting 350+ drugs of abuse, prescription drugs, and other substances by liquid chromatography with quadrupole time of flight mass spectrometry (LC-QToF-MS). Confirmation analyses of most analytes were undertaken by liquid chromatography with triple quadrupole mass spectrometry (LC-MS/MS) and LC-QToF-MS. PCP was confirmed via gas chromatography with mass spectrometry (GC-MS) using hydrogen as a carrier gas. The analytical method had (has) a 50 ng/mL quantitative reporting limit and a 40 ng/mL limit of detection. The analytical method was validated in accordance with internally developed protocols based on ABFT requirements, and AAFS-ASB guidelines and standards. Attributes assessed during method validation included linearity, precision and accuracy, matrix selectivity, internal standard recovery, and exogenous interferences.

From January 1, 2023, to May 1, 2025, the lab detected PCP in 83 postmortem toxicology cases across 8 states. Total number of blood cases screened was 47,364 across 40 different states in the USA. States included Arizona (1 positive case out of 1,733 total cases), Indiana (13 positive cases out of 11,558 total cases), Kansas (3 positive cases out of 1,729 total cases), Missouri (61 positive cases out of 4,672 total cases), Nebraska (1 case out of 2,250 total cases), New York (2 positive cases out of 207 total cases), Ohio (1 positive case out of 3,344 total cases), and Texas (1 positive case out of 310 total cases). The mean postmortem blood PCP concentration was 212 ng/mL and the median was 157 ng/mL. Other drugs or drug metabolites detected in these cases included delta-9-THC/delta-9-THC-COOH (26), cocaine/benzoylecgonine (23), nicotine/cotinine (23), methamphetamine (22), ethanol (21), and fentanyl/norfentanyl (19). PCP was detected as the lone substance in 11 cases. Excluding caffeine, nicotine, cotinine, naloxone, and amiodarone, PCP was detected as the sole substance of toxicological relevance in 16 cases. For all 83 cases, PCP blood concentrations ranged 52.5-973 ng/mL (mean, 212 ng/mL; median, 157 ng/mL). For the 16

cases where PCP was the lone substance of relevance, blood concentrations ranged 63.7-957 ng/mL (mean, 313 ng/mL; median, 258 ng/mL).

The prevalence of PCP seems to be geographically dependent in the USA. The vast majority of our positive casework was from Missouri. While most of the focus of the last two decades has been on the rapid emergence of various novel psychoactive substances (NPS), it is important to remember that the older, more classical drugs of abuse still exist, and it is prudent that the postmortem toxicology laboratory include them in every comprehensive toxicological analysis.

P-055

The Important Contribution of Glucuronide Metabolite Hydrolysis to Detection and Quantitation in Urine Drug Testing by LC-MS/MS

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Abstract

Introduction: Phase 2 drug metabolism, catalyzed by UDP-glucuronosyltransferase (UGT) isoforms, may contribute significantly to urinary elimination of many drugs and their phase 1 metabolites. While direct testing for glucuronidated metabolites is not routinely performed in definitive testing for large panels of drugs and their metabolites, glucuronidase hydrolysis is widely used to test for combined free and glucuronidated forms. We recently reported drug-prevalence findings by definitive urine drug testing in 400 emergency department patients suspected of drug misuse (JMSACL 2025 37;16-27). A large panel of drugs and their phase 1 metabolites were tested with use of glucuronidase hydrolysis, and over 1500 individual drugs and metabolites were identified. Further studies have been conducted to determine the contribution of hydrolysis to positive drug testing for these patients.

Objectives: A previous study of drug-use in 400 emergency department patients has been extended. We have co-analyzed samples with and without glucuronidase hydrolysis and have determined the impact of hydrolysis on drug detection and quantitation for a large panel of drugs and their phase 1 metabolites.

Methods: Definitive drug testing was performed on urine samples obtained from 400 patients presenting to the Albany Medical Center Emergency Department. The discarded, de-identified samples were obtained from 245 male and 155 female patients ranging in age from 18 to 89 years (mean 45 years). The study protocol was reviewed and authorized by the Institutional Review Board of the Albany Medical College. A panel of 110 analytes (drugs and their metabolites), made up of 104 abused drugs and psychiatric medications and six different cannabinoids (Δ9 THC, Δ8 THC, Δ9 cTHC, Δ8 cTHC, 11-hydroxy THC and CBD), was analyzed with and without hydrolysis using multiple developed LC-MS/MS methods.

Results: In the 360 patients with positive test results, a total of 1,866 specific drug and metabolite tests were positive with glucuronidase hydrolysis, representing positive findings for 76 of the 110 analytes in the panel. When the free form of the analytes was additionally determined without hydrolysis, the co-analysis showed that hydrolysis contributed to greater than 50% of the test response for a majority (39 out of 76) of analytes in the panel. The glucuronidated form of analyte was the predominant (>80%) contributors to quantitative results for testing in 24 of the 76 analytes in the panel, including drugs in the cannabinoid, benzodiazepine, opioid and anti-psychotic drug classes. Evaluation of detection sensitivity without hydrolysis revealed a decreasing detection rate for testing of the 32 panel analytes with greater than 60% glucuronidation, based on the co-analysis study. A 50%, or greater, false-negative rate without hydrolysis was found for testing within the cannabinoid, benzodiazepine, opioid and anti-psyschotic drug classes. Results for four analytes in the panel (cannabidiol, α-hydroxy alprazolam, 11-hydroxy THC and buprenorphine) showed a false negative rate of 100% without hydrolysis. The percentage of

analyte glucuronidation correlated directly with quantitative performance, and greater than 99% decrement in concentration was determined with quantitative results for 16 panel analytes when hydrolysis was not performed.

Discussion: With widespread use of glucuronidase hydrolysis in urine drug testing, our knowledge is limited regarding the relative contribution of glucuronidated metabolites to detection sensitivity and quantification performance in drug and phase 1 metabolite testing. This study shows that glucuronidated metabolites are major contributors to analyte detection and quantitation for a majority of analytes in the study panel. Detection rates fall precipitously without hydrolysis when the glucuronidated form exceeds 60%. Quantitative performance for many analytes is enhanced by hydrolysis in direct correlation with the ratio of glucuronidated to free analyte. It is concluded that glucuronide hydrolysis is a major contributor to analytical detection and quantitation for many drugs and phase 1 metabolites tested in routine forensic and clinical toxicology practice.

P-056

Homogeneous Enzyme Immunoassays for Fentanyl, Norfentanyl and Xylazine

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Abstract

Introduction: Immunoassays are the most common screening methodology for urine drug tests due to the high throughput and ease of integration into clinical chemistry platforms. The opioid epidemic accelerated the development and FDA clearance of several Fentanyl immunoassays to monitor compliance and identification of exposure in unprescribed abuse. Norfentanyl, a fentanyl metabolite, is an important component of identifying people who use fentanyl when urine is the specimen matrix. Norfentanyl is also an intermediate in the synthesis of fentanyl and frequently present in illicit fentanyl. With the inclusion of norfentanyl increasing the number of positive specimens by 42% over analyzing for fentanyl alone (Depriest A. et al, JAT 34(8), 444-449, 2010). Guidelines for federal workplace drug testing programs now include fentanyl in urine at a cutoff at 1 ng/ml with at least 5% cross reactivity to norfentanyl. Adulteration of fentanyl and other drugs with xylazine, an animal sedative, recently has been on the rise complicating patient treatment in emergent situations.

Objectives: Development of a fentanyl and norfentanyl urine drug test that meets federal workplace testing criteria of the 1 ng/ml cutoff for fentanyl with 5% cross-reactivity to norfentanyl. A xylazine immunoassay capable of detecting xylazine at a 10 ng/ml cutoff in urine samples.

Methods: The ARK™ Fentanyl Plus Assay and ARK™ Xylazine Assay are liquid-stable homogeneous enzyme immunoassays each consisting of two reagents, an antibody to the analyte and an enzyme (G6PD) labeled analyte that compete for the analyte. Binding of the analyte labeled enzyme conjugate to the antibody reduces enzyme activity. In the presence of analyte from the specimen, enzyme activity increases and is directly related to the drug concentration. Enzyme activity is measured spectrophotometrically as the change in absorbance at 340nm of the coenzyme NAD+ to NADH upon reduction. The performance characteristics of the ARK assays, including precision, spiked recovery, specificity, and method comparison to LC-MS/MS, were evaluated on the Beckman Coulter AU480 automated clinical chemistry analyzer.

Results: Qualitative precision analysis of the ARK Fentanyl Plus using histogram overlap exhibited no overlap between the cutoff and control levels at both ±25% and ±50% of the 1 ng/mL fentanyl cutoff. The major metabolite, norfentanyl, showed an approximate equivalence to the 1 ng/mL fentanyl cutoff at 1.1 ng/mL (90.1% cross-reactivity). Method correlation with LC-MS/MS using authentic urine samples showed excellent agreement with a specificity of 100% and sensitivity of 100% (50 positives and 50 negatives). The ARK Xylazine Assay showed acceptable precision, with %CVs ranging from 5.8% to 8.7% in semi-quantitative mode and no overlap between cutoff (10 ng/mL) and ±50% control levels (5 ng/mL and 15 ng/mL) in a histogram overlap analysis. Spiked xylazine samples spanning the semi-quantitative assay range up to 500 ng/mL recovered between 87.7% and 100.6% of the spiked levels. Two of the major metabolites, 3-hydroxyxylazine and 4- hydroxy xylazine, were detected as positive at concentrations of 9.1 ng/mL (109.8% cross-reactivity) and 18.9 ng/mL (53.0% cross-reactivity), respectively. Concentrations determined by the ARK Xylazine Assay of 126 patient urine samples containing a combination of fentanyl, norfentanyl,

and xylazine were in excellent correlation with that obtained by LC-MS/MS.

Discussion: The ARK™ Fentanyl Plus Assay provides a highly sensitive and efficient method for detecting fentanyl and its major metabolite, norfentanyl, in human urine. With the addition of the ARK™ Xylazine Assay, it enables a high-throughput analysis of all three analytes across a wide range of clinical chemistry analyzers, making it a valuable tool for both clinical and forensic toxicology applications.

Conflict of Interest

All the authors are employees of ARK Diagnostics Inc.

P-057

Detection and Validation of PCP, Ketamine, Analogs and Metabolites in Blood using LC-MS/MS

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Abstract

Introduction: In recent years, novel psychoactive substances (NPS) have continued to be produced and sold throughout the world. With constantly evolving drug trends, detection and identification of NPS in forensic casework can be difficult. For the last five quarterly reports published by the Center for Forensic Science Research and Education (CFSRE), NPS stimulants and hallucinogens accounted for an average of 14% of NPS drug cases in the United States. Specifically, several analogs of phencyclidine (PCP) and ketamine have been increasing in prevalence to include analytes such as 3-methyl-PCP, 3-methoxy-PCP, and 2-fluoro-2-oxo-PCE. As a result, new toxicological methodologies need to be developed and validated for the identification of these substances in casework.

Objectives: The purpose of this study was to develop and validate an extraction and detection method for PCP, ketamine, and selected analogs and metabolites in blood using liquid chromatography tandem mass spectrometry (LC-MS/MS).

Methods: A quantitative method was developed for ten drugs: PCP, 3-methoxy PCP, 3-methyl PCP, ketamine, norketamine, deschloroketmaine, deschloro-*N*-ethyl ketamine, 2-fluoro-2-oxo-PCE, fluorexetamine, and methoxetamine. PCP-d_5, ketamine-d_4, and norketamine-d_4 were used as internal standards.

Using whole, preserved bovine blood, a liquid-liquid extraction protocol was optimized for sample preparation. Briefly, the extraction began with fortification of 0.5 mL blood with analytes and internal standards followed by the addition of 1.5 mL 10 mM borate buffer (pH ~ 9) and 3 mL *N*-butyl chloride extraction solvent, with vortexing between each addition. Samples were rocked for 5 minutes and centrifuged at 4200 RPM for 10 minutes. The organic layer was transferred to conical tubes and dried under nitrogen (40°C) for 15 minutes. Samples were reconstituted with 50 µL of starting mobile phase and transferred to high recovery autosampler vials.

For chromatographic separation and detection, an LC-MS/MS method was optimized on an Agilent 1290 Infinity II LC coupled with an Agilent 6475 LC/TQ. Instrument parameters such as capillary voltage, gas flow, gas temperature, nebulizer, nozzle voltage, sheath gas flow, and sheath gas temperature were optimized. Mobile phase A consisted of 0.1% formic acid in deionized water and mobile phase B consisted of 0.1% formic acid in acetonitrile. An Agilent Poroshell 120 EC-C18 (2.1 x 100 mm, 2.7 µm) column with a matching guard was utilized for chromatographic separation. All analytes were separated, including the isomers 2-fluoro-2-oxo-PCE and fluorexetamine, with the following mobile phase gradient: 0.00 minutes (15 % B), 0.50 minutes (15% B), 2.50 minutes (22.5% B), 4.25 minutes (65% B), 5.25 minutes (90% B), 6.25 minutes (90% B), and 8.50 minutes (15% B).

The method was validated according to ANSI/ASB Standard 036, and the following parameters were assessed: calibration model, limit of detection, limit of quantitation, bias, precision, carryover, ionization suppression/enhancement and possible interferences.

Results: The described method was successfully validated according to ASB 036. All analytes were validated with a quadratic 1/x weighted model, with a calibration range of 0.5 – 500 ng/mL. Bias and precision were evaluated at three concentrations (1.5, 125, and 400 ng/mL) and controls met all appropriate acceptance criteria. Matrix effects did not exceed ±20% and were deemed acceptable. The limits of detection and limits of quantitation were 0.1 ng/mL and 0.5 ng/mL, respectively, for all analytes. No carryover was observed following a 500 ng/mL calibrator. No interferences from the matrix, internal standard, commonly encountered drugs of abuse and prescription drugs were detected.

Discussion: This study presents a validated LC-MS/MS method for the detection of PCP, ketamine, metabolites, and selected emerging analogs in blood, offering a practical tool for forensic toxicology laboratories. This method demonstrated acceptable quantitative performance using a simple liquid-liquid extraction and can support the identification of emerging NPS substances related to PCP and ketamine in forensic toxicology casework.

P-058

Sting Operation: Identification of Grayanotoxins in "Mad Honey" via Direct Analysis in Real Time Time-of-Flight Mass Spectrometry

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Abstract

Introduction: Grayanotoxins are a group of naturally occurring diterpenoid compounds found primarily in plants of some species of the Ericaceae (Rhododendron) family. These neurotoxic compounds exert their effects by binding to voltage-gated sodium channels and disrupting normal nerve and muscle function. Himalayan giant honeybees in the Hindu Kush region collect nectar from a specific rhododendron species, Rhododendron ponticum, which contains grayanotoxins. These grayanotoxins are then incorporated into the honey. The ingestion of "mad honey" has been used for its hallucinogenic effects, but can have adverse effects (e.g., gastrointestinal distress, bradycardia, severe hypotension, neurological impairment). In the last decade, "mad honey" has become more prevalent in media and pop culture, and. It is readily available from online retailers such as Etsy and Amazon. Intoxications and poisonings have been reported across the United States. It is not controlled at either the federal or state level.

Objectives: The purpose of this experiment was to identify grayanotoxins in "mad honey" samples using Direct Analysis in Real Time Time-of-Flight Mass Spectrometry (DART-ToF-MS).

Methods/Results: Five honey samples marketed as "mad honey" or "Himalayan honey" from various online retailers were purchased in 2019, 2021, and 2022. Both raw and extracted samples were analyzed. The samples were extracted following a previously published method. In brief, 5 grams of sample and 5 mL of water were vortexed, extracted into 25 mL of methanol followed by 25 mL of ethanol, twice. An Oasis HLB SPE cartridge conditioned with ethanol and water was used for final sample clean-up and concentration.

The extracted samples and primary reference material of grayanotoxin III (Cayman Chemical) were analyzed using a DART-TOF-MS method. The instrument was operated in positive-ion mode with a helium stream of 2.0 L/min at 350°C. The discharge electrode needle was set to 150 V and the ion guide peak voltage to 400 V. Orifice 2 was set to 5 V, and the ring lens was set to 3 V. Orifice 1 was operated at 20 V. The mass range was measured from 40 to 1,100 Da. The data was analyzed using the average, background subtracted and centroid mass instrument calibrator PEG 600. A positive identification was made when the protonated molecular ion ([M+H]+) of grayanotoxin III at (371.3196 m/z ± 5 millimass units) was identified.

Discussion: The DART-MS method was unable to identify grayanotoxin III in the unextracted samples, possibly due to the high abundance of sugars and other honey components. However, grayanotoxin III was successfully identified in all extracted samples. Some of the products were purchased in 2019, suggesting grayanotoxin III stability in honey. Although "mad honey" is not produced in the United States, its rise in popular culture and media, along with online accessibility, dictate the need to raise awareness.

Conflict of Interest

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P-059

From Bud to Puff: Characterizing Cannabinoids in E-Cigarette Emissions

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Abstract

Introduction: The medical marijuana (regulated) market has seen rapid growth, with increasing availability of vaporized products. Manufacturing and quality assurance practices remain problematic, even within the regulated market. Many products have been identified with incorrect or altogether missing ingredient labels. Current research into the aerosolization of cannabis vape constituents is limited, especially surrounding device consistency and dosages delivered. Understanding both aerosolization efficiency and the formation and/or conversion of cannabinoids during vaporization is critical for assessing potential health risks.

Objectives: The purpose of this experiment was to determine the chemical constituents of a medical marijuana vape formulation and the corresponding aerosol, and to evaluate the efficiency and consistency of the device.

Methods: A Bloom Surf Pineapple Express HYBRID 1g Disposable vape, was purchased from a medical marijuana shop in Richmond, Virginia. The device was labeled to contain 87.98% delta-9-tetrahydrocannabinol (Δ9-THC), 1.49% cannabigerol (CBG), 0.54% cannabinol (CBN), and terpenes. Capture of the aerosol (n=5) was performed using an in-house impinger trapping system. Each trial consisted of 10 five-second puffs drawn via a vacuum into an impinger containing Type 1 Water. The device was weighed before and after each puffing session to determine the mass of product aerosolized. After aerosol collection, the vape was opened and a portion of the product was diluted in methanol for analysis.

Vape constituents were determined by untargeted analysis of a methanolic dilution of the product using a Shimadzu QP-2020 gas chromatograph with mass spectrometer (GC-MS). Primary reference materials and spectral libraries (NIST, SWGDRUG) were used for identification. The aerosol was not evaluated by GC-MS due to the aerosol collection solvent being water. Cannabinoids were identified and quantitated in the product and corresponding aerosol were analyzed using a Shimadzu LCMS-8050 liquid chromatograph with tandem mass spectrometer (LC-MS/MS).

Results: Cannabinoids, Δ8-THC, Δ9-THC, CBG, CBN, CBT, Δ9-tetrahydrocannabutol (Δ9-THCB), and terpenes, d-limonene, α-terpineol, β-caryophyllene, and α-humulene were identified in the vape formulation. The weight per volume (w/v) was extrapolated to 61.0 ± 2.2% Δ9-THC, 1.18 ± 0.14% CBG, 0.80 ± 0.05% CBN, 1.01 ± 0.008% Δ8-THC, 0.149 ± 0.02% Δ9-THCB.

Δ9-THC, CBG, CBN, Δ8-THC and Δ9-THCB, were identified in the aerosol. When extrapolated to a single 5-second puff, the Δ9-THC was 1.7±0.1 mg; the CBG was 0.032±0.003 mg; CBN was 0.020±0.003 mg; Δ9-THCB was 0.0055 ±0.0004 mg; and Δ8-THC was 0.013±0.002 mg per puff. The average mass of e-liquid aerosolized in 10 puffs was 24.17 ± 3.7 mg.

Discussion: All vape concentrate constituents had greater than 20% difference from the labelled concentration. The presence of the terpenes d-limonene, α-terpineol, β-caryophyllene, and α-humulene matches the labelled profile of the product. However, the identification of CBT, Δ8-THC, and Δ9-THCB raises concern about product labeling transparency and verification in the regulated marijuana market. The similar ratio of cannabinoid components between product and aerosol indicates the efficiency of these compounds to aerosolize, but aerosol results did not align with packaging claims of receiving 9.36 mg of THC per 5-second puff. Despite this, >1 mg of THC/puff will still result in a psychoactive effect. Aerosol data showed consistent delivery between trials, suggesting the device was consistent. Evaluating aerosolization of cannabis vaping product is critical for ensuring dosing is consistent between puffs and label claims are accurate.

Conflict of Interest

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P-060

The Prevalence of Designer Benzodiazepines in Tarrant County, TX

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Abstract

Introduction: Designer benzodiazepines (DBZDs) are a subclass of NPS that suppress the central nervous system similar to the more common licit benzodiazepines. They are often mixed with opioids and stimulants then pressed into illicit pharmaceutical pills. Benzodiazepine receptor agonists work through $GABA_{(A)}$ to promote sleep by inhibiting the brainstem's dopaminergic arousal pathway. Increased GABA levels help calm and relax a person by reducing anxiety and inducing sleep. One of the main problems with benzodiazepines and DBZDs is withdrawal. A sudden decrease in GABA levels may cause severe symptoms including seizures, sound and touch sensitivity, numbness in extremities, suicidal ideations, psychosis, altered perception, sensitivity to light, and hallucinations. For these reasons DBZDs are important in both driving under the influence of drug(s) (DUID) and postmortem toxicology cases.

Objectives: To present the prevalence of DBZDs in DUID and postmortem cases from 2020 through July 2025 from the Tarrant County Medical Examiner's Office (TCME) in Fort Worth, TX.

Methods: The 29 DBZDs evaluated for prevalence statistics were 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 (LC-QTOF-MS) for confirmation. The LC-QTOF-MS screens and confirms approximately 400 compounds that encompass illicit, prescription, over the counter, and novel psychoactive substances (including the 29 DBZDs). For quantitative confirmation analysis, only flualprazolam and etizolam have been validated. A solid phase extraction procedure coupled with LC/MS/MS was performed that required 400 µL of specimen and yielded a limit of quantitation (LOQ) of 5 ng/mL. All other DBZDs are reported qualitatively.

Results: From 2020 to present day, 26,636 cases have been analysed by TCME with 584 (2%) confirmed as DBZDs positive. A trend of flualprazolam being the most prevalent designer benzodiazepine in 2020 and 2021 to bromazolam being the most prevalent in 2024 and 2025 at TCME was noted. Approximately eighty percent of NPS cases were coupled with opioids (33.2%) and/or cannabinoids (59.4%). In 3.8 % of cases, DBZDs were the sole positive finding.

Discussion: It was surprising to observe the prevalence of flualprazolam decrease over time, from 3.7% of all cases in 2020 to 0% in 2025. Currently, bromazolam is the most observed DBZD in the Tarrant County area at 1.0% of all cases. Overall, there has been a decrease in DBZDs confirmed cases from 4.7% (2020) to 1.3% (2025). This decrease could be due to new DBZDs that are not detected by the current laboratory methods. In the future, TCME will continue to monitor and confirm cases of DBZDs by expanding the current designer benzodiazepine methodology to include new compounds as they are synthesized and standards become available.

Keywords

Designer benzodiazepines, Antemortem, Postmortem, Prevalence

P-061

Accelerating Health Risk Assessment of PFAS Exposure in 10 Seconds: A Toxicological Approach Using LDTD-MS/MS

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Abstract

Introduction: Per- and polyfluoroalkyl substances (PFAS), often referred to as 'forever chemicals,' are an increasingly concerning class of emerging contaminants due to their widespread presence in everyday environments and their potential health risks. Quantifying PFAS levels in individuals is a key step toward advancing exposome research, with the goal of identifying and reducing population-level exposure sources. To enable more effective monitoring, it is essential to develop rapid and high-throughput screening methods that reduce the analytical burden on laboratories when assessing PFAS.

Objectives: To reduce laboratory workload and enable efficient biomonitoring of PFAS, ultimately supporting risk assessment and public health decision-making by developing a simple, rapid and automated sample preparation method based on liquid–liquid extraction, combined with high-throughput analysis method using LDTD-MS/MS.

Methods: The calibration curve and quality control samples were prepared in an artificial matrix consisting of 20 mg/mL bovine serum albumin (BSA) in phosphate-buffered saline (PBS). Nine common PFAS compounds were evaluated using this method, which is based on functional groups rather than PFAS subclasses. The compounds included those with sulfonic acid function (PFOS, PFBS, PFHxS, and 6:2-FTS) and carboxylic acid function (PFOA, PFNA, PFHxA, PFBA, and HFPO-DA). The calibration range extended from 1 to 20 ng/mL.

Automated extractions were performed as follows: Plasma samples (100 µL) were transferred into 12X75 mm borosilicate glass tubes, and 10 µL of internal standard solution was added. Samples were mixed using a Vortex for 30 seconds at 1100 rpm. Acidification was performed by adding 30 µL of 4 N hydrochloric acid, followed by dilution with 300 µL of a 20 mg/mL BSA solution in PBS. The mixture was mixed again for 30 seconds at 1100 rpm. Liquid–liquid extraction was carried out by adding 600 µL of an extraction solvent composed of chloroform and acetonitrile (1:1, v/v). After mixing for 1 minute at 1100 rpm, the samples were centrifuged at 5000 rpm for 5 minutes. The 200 µL lower organic phase was carefully transferred to a 6X31 mm borosilicate glass tube, mixed with 24 µL of evaporation solution and evaporated to dryness at 40°C. The dry residue was reconstituted with 100 µL of REC-1. For LDTD-MS/MS, 5 µL of the extracted sample was applied to a LazWell plate and dried at 40 °C for 5 min (sulfonates). For carboxylic acids, 2.5 µL of REC-2 was added to the plate, followed by 5 µL of the extracted sample, and dried under the same conditions. REC-1 and REC-2 serve as reconstitution and desorption solutions, specifically formulated to enhance analyte desorption and ionization. The inclusion of specific additives in the desorption mixture has been shown to improve the efficiency of analyte desorption. This principle is consistent with findings reported by Dion-Fortier et al. (2019) and Gravel et al. (2019).

LDTD-MS/MS analysis was performed using a Luxon Ion Source T-960 coupled to a Thermo TSQ-Altis plus mass spectrometer. The instrument was operated in negative APCI mode. The laser desorption pattern was a 6 second ramp to 100%, 4 second hold, then return to 0% for sulfonates;

and a 6 second ramp to 65%, 1 second hold, then return to 0% for carboxylic acids. The carrier gas flow was maintained at 6 L/min. A specific multiple reaction monitoring (MRM) transition was used for the screening.

Results: For this method, the coefficient of determination must be equal or higher than 0.99. Preliminary results shows that the determination coefficients (R^2) obtained were greater than 0.990 for both PFAS (carboxylic acids and sulfonates) groups across six analytical runs (6 calibrations points). For screening, the precision and accuracy criteria require a coefficient of variation (CV) of ≤20%, and a deviation of ≤20% from the expected value (%Bias). For PFAS (sulfonates), the inter-run precision yielded a CV lower than 11.5%, and the Bias lower than 6.5%. For PFAS (carboxylic acids), CV obtained was lower than 11.5% and accuracy lower than 3.3% for inter-run values (n=18). A cross-validation study was conducted with real plasma samples using LC-MS/MS. The R^2 obtained was higher than 0.95 between the two technologies.

Discussion: LDTD combined with a TSQ Altis plus mass spectrometer system allows ultra-fast (10 seconds per sample) screening of PFAS in human plasma samples.

Conflict of Interest

2) Salary / Consultant

P-062

Assessing a New Recombinant β-glucuronidase Enzyme for the Analysis of Cannabinoids in Urine

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Abstract

Introduction: Cannabis is considered one of the most widely used substances worldwide and is the focus of considerable research and legal debate. The metabolites of tetrahydrocannabinol (THC) are extensively conjugated via glucuronidation. Therefore, alkaline, enzymatic, and/or tandem hydrolysis may be required for complete and definitive analysis of parent drugs and metabolites in urine specimens. Recently, an increasing number of unregulated hemp-based products containing cannabidiol (CBD), and/or Δ^8-THC have become widely available in the US. The importance of optimizing sample preparation and hydrolysis of conjugated molecules has been described extensively in the literature. A lack of hydrolysis efficiency may result in poor recovery of the target compounds and unreliable results. In addition, enabling simple, rapid, and cost-effective testing is an evolving need in clinical and forensic toxicology.

Objectives: The aim of this study was to evaluate the enzymatic efficiency of recombinant β-glucuronidase enzyme solutions for high-throughput analysis of cannabinoids in authentic urine specimens. The incubation was performed at room temperature.

Methods: The new prototype recombinant β-glucuronidases (*E1, E2, and E3*) and the commercially available BGTurbo® and B-One® were obtained from Finden KURA Biotech (Atlanta, GA, USA). Authentic urine samples were obtained from patients enrolled in a study assessing the prevalence of marijuana/CBD use. The study protocol including the collection of urine samples was approved by the University of Florida Institution Review Board (IRB202300712). The sample preparation consisted of a simple dilution and filtration of the samples followed by the LC-MS/MS analysis as described in Ballotari et al. [1]. In this study, blank urine samples were fortified with Δ^8-THC, Δ^9-THC, 11-OH-Δ^8-THC, 11-OH-Δ^9-THC, Δ^8-THC-COOH, Δ^9-THC-COOH, CBD, 7-COOH-CBD, CBG, and CBN in triplicate at 30 ng/mL, and the hydrolysis step modified (temperature/incubation time) according to the prototype formulation. A volume of 0.3 mL of the enzyme was added to 0.5 mL of the sample, followed by an incubation time of 15 min at room temperature (20-25°C). The hydrolytic activity of each enzymatic reagent in the cleavage of the studied cannabinoids at room temperature was initially studied in terms of analyte recovery from blank matrix and ion suppression/enhancement (matrix effect). Authentic urine specimens were assessed quantitatively.

Results: Following the modified sample preparation and hydrolysis, the results indicated minimal matrix effect and acceptable extraction recovery for all the analytes upon the use of different enzymes and ranged from 84 to 105% and 74 to 130%, respectively. Two authentic urine specimens were also qualitatively analyzed in triplicate. A 10-fold difference in terms of peak area was observed for the prototype formulation *E1* when compared to B-One®, in particular for the CBD metabolite 7-COOH-CBD. Therefore, a second set of experiments comparing the validated protocol described in Ballotari et al. with the new enzymatic hydrolysis using the β-glucuronidase *E1* was performed. Authentic urine specimens (n=4) previously tested positive for CBD were

quantitatively analyzed. A retention time shift was observed for 7-COOH-CBD and the ion ratio of the qualifier ions did not pass the established criteria for quantitative analysis. The variability of the quantitative values determined between methods was minimal for the other detected cannabinoids.

Discussion: Although tandem enzyme-alkaline hydrolysis achieved optimum glucuronide de-conjugation and cleavage of the ether and ester bonds, the hydrolysis requires longer incubation and more manual workflows. The temperature, the enzyme:sample ratio, and the class and concentration of the target analytes are critical and highly specific while using β-glucuronidases. The faster hydrolysis at room temperature, promoted by the newly developed recombinant enzymes, is an asset for laboratories aiming to increase the number of processed samples, minimizing the loss of analytical performance, and yield. The unexpected preliminary results, obtained in this study for 7-COOH-CBD, could be due to a cleavage activity promoted by the enzyme to other compounds present in the matrix as products of metabolism and/or interferences. Further experiments are needed to verify the enzymatic efficiency of the enzymes for analysis at room temperature of cannabinoids in authentic urine specimens.

References

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Conflict of Interest

The authors have no conflicts of interest to declare. The prototype recombinant β-glucuronidase solutions were provided by Finden® KURA Biotech (Atlanta, GA, USA).

P-063

The Reemergence of Carfentanil in the State of Florida: A Recent Case Series

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Abstract

Introduction: The opioid epidemic in the United States (U.S.) is currently driven by synthetic opioid use, principally fentanyl and its analogs. Over the past decade, many derivatives, and fentanyl-related substances have been identified in the illicit drug market. This has led the U.S. Drug Enforcement Administration (DEA) to schedule all illicitly manufactured fentanyl and related products based on core structure. Carfentanil is a potent analog of fentanyl and a serious threat to public health. In the U.S., carfentanil was first identified in decedents in 2016 predominantly in the states of Florida and Ohio. Following the adoption of control measures in the U.S. and China, the prevalence of carfentanil in postmortem casework dropped precipitously. However, a resurgence in the prevalence of carfentanil has been reported throughout the U.S.

Objectives: This study aims to characterize the recent emergence of carfentanil in decedents in the state of Florida.

Methods: Blood and urine specimens obtained from decedents were submitted to the forensic toxicology laboratory for drug analysis. The analysis of carfentanil in blood was achieved using liquid chromatography-tandem mass spectrometry (LC-MS/MS) performed at NMS Labs (designer opioids assay) with a lower limit of quantitation (LLOQ) of 0.050 ng/mL. Urine specimens were qualitatively screened utilizing liquid chromatography-quadrupole time-of-flight mass spectrometry (LC-QTOF-MS) with a detection limit of 0.500 ng/mL.

Results: Approximately 2500 cases from five medical examiner districts were submitted to the forensic toxicology laboratory for drug analysis. Carfentanil was detected and quantified in two antemortem blood specimens and 17 postmortem blood specimens. The age of the decedents ranged from 28 to 63 years (median: 41.0 years; mean: 41.3 years). All of the decedents were Caucasian, with males being the majority (73.7%). The concentration range of carfentanil in the postmortem blood specimens was 0.10-2.20 ng/mL (median: 0.40 ng/mL; mean ± S.D.: 0.65 ± 0.58 ng/mL), while in the two antemortem blood specimens carfentanil was detected at a concentration of 0.058 ng/mL and 0.23 ng/mL, respectively. Fentanyl was quantified with carfentanil in nine blood specimens with a concentration range of 3.1-55 ng/mL (median: 14.1 ng/mL; mean ± S.D.: 19.3 ± 17.3 ng/mL) and detected in one case below the lower limit of quantitation (LLOQ, 2.5 ng/mL). The fentanyl analog para-fluorofentanyl (p-FF) was also detected in six postmortem blood specimens with a concentration range of 0.07-0.30 ng/mL (median: 0.11 ng/mL; mean ± S.D.: 0.14 ± 0.08 ng/mL), while xylazine was identified in the blood of two decedents but not quantified. Polysubstance use associated with carfentanil was also observed. Stimulants (57.9%), other opioids (15.8%), benzodiazepines (10.5%), NPS benzodiazepines (5.3%), cannabinoids (78.9%), ethanol (10.5%), and other drugs (47.4%) were identified in the decedents. Based upon the toxicology findings, the medical examiners certified the cause of death as drug toxicity with the manner of death deemed accidental in a majority of the cases (89.5%), with only one suicide.

Discussion: This study aimed to promote increased awareness regarding the re-appearance of carfentanil in forensic toxicology postmortem casework in the state of Florida. The concentrations of carfentanil detected in the postmortem blood specimens were comparable to those previously reported in the state and the literature. Carfentanil was identified either with or without fentanyl and/or other fentanyl analogs in the authentic specimens. These findings reflect an increase in the prevalence of carfentanil in the illicit drug supply and pose a significant public health risk of non-fatal and fatal overdose.

P-064

The Science of Connection: Analyzing the SOFT Professional Mentoring Program's Journey

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Abstract

Introduction: The SOFT Professional Mentoring Program (PMP) Committee was formed in 2020 to provide opportunities for sharing one-on-one career advice, developing leadership skills, and promoting the transfer of knowledge through mentee and mentor pairs in the toxicology community. In addition to matching pairs with specific needs and skill sets, the PMP committee also provides various resources and activities to help facilitate the success of the pairs throughout the year as they work to meet their goals. To make sure each new year is successful for the pairs, an annual survey is sent to participants to assess committee-defined mentoring benefits, track progress between pairs, establish measurable outcomes, and target program improvements. The SOFT-PMP is a data-driven mentoring program that has become an essential component of the forensic toxicology community.

Objectives: To evaluate the SOFT-PMP's benefits, impact and mentor/mentee outcomes in 2024 and assess 2020-2024 trends.

Methods: Feedback on participant achievements (goals assessment), and program structure/content (program value assessment) were collected via participant surveys at the end of each mentoring program year from 2020-2024. There were some minor differences in the surveys from year to year. Feedback gauged assessment of satisfaction, value and interest in the overall program, with focus areas including professional goals and program benefits.

Results: The percentage of participants who completed the survey was 52% in 2024, 32% in 2023, 51% in 2022, 76% in 2021, and 66% in 2020. Since its inaugural year in 2020, the program has continued to meet and exceed the survey participants' original expectations: 91% in 2024, 89% in 2023, 92% in 2022, 90% in 2021, and 88% in 2020. Program participation saw less new pairs in 2024 but more returning pairs than in 2023. Since its inception, the program has shown an impact in the main areas of SOFT engagement, career advancement, and development of interpersonal/leadership skills, with the latter demonstrating increased interest into 2024. Furthermore, a 4th area, technical development, is starting to emerge and may be the new trend in coming years. The most significant professional outcome for 2024 was interpersonal/leadership skills at 68% with technical development moving up to the second most significant outcome at 63%. Data from 2024 shows that transfer of knowledge and expanding professional networks continue to rank as the top benefits (>80%) participants obtained from the program. Leadership development has increased in value as a major benefit matching expanding professional networks

in 2024 (86%). In 2024, ToxTalk was the second highest valued activity at 60% with the virtual kickoff webinar becoming the most valued activity at 71%. Among the different activities organized from 2020 to 2024, the five most valued (>60%) were virtual kickoff webinar in 2024, resources published in ToxTalk in 2023, imposter syndrome webinar in 2022, monthly resources emails in 2021, and the webinar in 2020.

Discussion: The PMP has seen strong participation with a 5-year average program feedback rate of 55.4%. A major challenge observed is a failure to launch or sustain the partnership, often due to communication issues or time constraints. Survey data allowed for the expansion of the virtual kickoff webinar to allow pairs to start strong with setting goals and to encourage them to foster their relationship with the help of program resources. Of those providing program feedback, transfer of knowledge, expanding professional networks, and leadership development continue to be major benefits to participants. As survey data is analyzed showing increased value of leadership development alongside decreased participation in activities despite indicated interest, modifications have been made in 2025 to allow historically valuable activities to better fit the needs of the mentoring community.

P-065

CANTERA™: AI-Driven Enzyme Development for Enhanced Opioid Metabolite Hydrolysis in Toxicology

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Abstract

Introduction: Morphine and codeine are commonly prescribed opioids monitored in urine drug testing to assess therapeutic and illicit use. Codeine is primarily metabolized to codeine-6-glucuronide (C6G), a key marker of intake, and partly to morphine, which further forms morphine-3-glucuronide (M3G) and morphine-6-glucuronide (M6G). Since morphine metabolites can result from codeine use, their presence alone may not confirm direct morphine or heroin intake. Interpreting the ratios of C6G, M3G, and M6G is therefore essential for distinguishing legitimate use from potential misuse.

To support accurate testing of these metabolites, we employed CANTERA™, our cutting-edge platform merging classic bioinformatics and AI-based tools, enabling a rapid process from protein discovery and engineering to enzyme expression. Special emphasis was placed on developing a novel β-glucuronidase (beta-glucuronidase) formulation enhancing the hydrolysis of known opioid metabolites, described as hard-to-cleave, such as C6G, M6G, and M3G, addressing common challenges in workflows incorporating enzymatic hydrolysis, including prolonged incubation times, elevated enzyme concentrations, and the need for heating or chemical additives.

Objectives: To develop and validate novel enzymatic reagents that efficiently hydrolyze opioid glucuronides in toxicology settings, employing the CANTERA™ protein engineering platform, for protein discovery, modelling, and experimental validation.

Methods: Beta-glucuronidase enzyme candidates, naturally occurring and engineered, for toxicology applications were identified using CANTERA™, Kura Biotech's proprietary protein engineering platform. This system integrates in silico simulations, AI-based tools, molecular docking, and targeted experiments to screen a large library of enzyme variants against the mentioned opioid substrates, M6G, C6G, and M3G. These tools enable the theoretical analysis of the active site of an enzyme and the potential substrate based on the proximity to the reaction site. Following several computational iterations, top candidates were selected based on predicted binding affinity and reaction feasibility. The selected naturally occurring and engineered candidates were expressed, purified, and formulated into enzymatic reagents (E1, E2, and E3).

Wet lab validation included assessment of the hydrolysis performance under mild conditions (no external heating and short incubation time) of phenolphthalein β-D-glucuronide (PPG), a model substrate for glucuronidase enzymes, and the target opioid substrates, M6G, C6G, and M3G. Formulation buffer assessment was conducted with a DoE approach to optimize pH and salt concentration for enzyme stability. Substrate conversion and enzymatic activity were quantified using a cost-effective HPLC method. All results were benchmarked against the wild-type *Brachyspira pilosicoli* β-glucuronidase (*Bp*WT) to evaluate hydrolytic efficiency and potential for routine toxicology applications.

Results: *The CANTERA platform* identified 1492 beta-glucuronidase variants. Of these, 29 exhibited promising hydrolytic activity against hard-to-cleave opioid metabolites with “in silico” docking studies, enabling a broad phylogenetic profile coverage. Expression and purification of selected candidates confirmed that five variants showed both strong expression levels and high hydrolytic activity.

The selected top enzymatic variants, which represent a significant step forward and introduce inventive enzymatic features not found in known sequences or commercial products, were further evaluated under varying pH conditions and buffer systems. Their stability profiles revealed optimal pH ranges and robust performance, positioning the three enzymatic reagents as strong candidates for analytical workflows.

Drug hydrolysis showed that after just 5 minutes, E1 and E2 hydrolyzed over 80% of the M3G and M6G substrates; in contrast, BpWT presents the same performance towards M3G but close to 40% of M6G. At 15 minutes, the 3 formulated enzymatic reagents significantly increased their activity, achieving over 95% hydrolysis across all tested substrates; meanwhile, BpWT presented roughly 40% hydrolysis of M6G and C6G.

Discussion: The new enzymatic reagents exhibited enhanced hydrolytic activity towards the targeted "hard to cleave" glucuronide-conjugated opioids compared to the *Bp*WT enzyme, in addition to an extended pH range, and organic solvent compatibility.

The formulation optimization evidenced that the effect of buffer identity and concentration, reaction pH, and presence of salts significantly influence the activity and stability of the selected beta-glucuronidase enzymes.

The use of our *CANTERA*™ Platform enables Kura Biotech to develop proprietary enzymes with desired features and successfully transform them into enzymatic solutions.

Conflict of Interest

2

P-066

Relative Concentration Distributions of Clonazepam and 7-Aminoclonazepam in a Large-Scale Oral Fluid Study

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Abstract

Introduction: Oral fluid (OF) is emerging as a preferred alternative to urine in drug testing due to its ease of collection, lower risk of tampering, and enhanced ability to detect recent drug use. However, lower concentrations of drugs and different metabolic profiles have been observed in OF as compared to urine, creating a challenge for accurate detection of drugs of abuse such as benzodiazepines (benzos). Benzos are a drug class characterized as weakly acidic and highly protein-bound, which results in lower concentrations of drug detected in OF. Clonazepam (Clon), a frequently prescribed benzo, is commonly abused because of its high potency. 7-aminoclonazepam (7AC), a metabolite of Clon, is often the predominant analyte detected in urine to indicate Clon use. Less is known about the detection profile of Clon and 7AC in OF because most OF testing includes only the parent compound, Clon. However, some OF studies suggest that both 7AC and Clon be included in OF testing panels for Clon use based on observations of higher 7AC concentrations than Clon concentrations in OF samples. In this study, 2,391 OF sample results that were positive for Clon and/or 7AC were analyzed to evaluate the most effective biomarkers for detecting Clon use in OF.

Objectives: To evaluate the efficiency of Clon use identification in OF by comparing Clon results alone to those that include Clon and/or 7AC.

Methods: Clinical specimens used in this study were submitted to Quest Diagnostics for OF benzos testing and de-identified results were retrospectively analyzed. OF specimens were collected with a standard Quantisal™ container followed by sample preparation using solid phase extraction on a Hamilton automated liquid handling system. The extracted sample mixture was loaded for injection onto a Sciex QTRAP 6500 tandem mass spectrometer coupled with a Shimadzu high performance liquid chromatography (HPLC) system. The OF assay was fully validated under CLIA regulations for lab developed tests. The benzo panel included midazolam, lorazepam, triazolam, flunitrazepam, alprazolam, diazepam, clonazepam, oxazepam, temazepam, nordiazepam, flurazepam, chlordiazepoxide, and 7AC. The clinically reportable range for all benzos (CRR) was 0.5 to 25 ng/mL.

Results: Retrospective analysis was performed in OF testing results and there were 2,391 positives for Clon and/or 7AC. Among these, 184 (8%) were positive for Clon only; 1,145 (48%) were positive for both Clon and 7AC; 1,062 specimens (44%) positive for 7AC only. These results suggest that 7AC should be included in OF testing for Clon use. Among the specimens that were positive for both Clon and 7AC, 1,014 specimens were within the CRR. The average 7AC/Clon concentration ratio was 5.4, supporting the overall prevalence of higher 7AC concentrations than Clon concentrations. However, the 7AC/Clon ratio varied widely (0.1 to 49.8), reflecting variation of pre-analytical factors including function of time from drug consumption to specimen collection and/or patient differences in Clon metabolism patterns.

Discussion: In this study, clinical OF specimen results were used to evaluate relative concentration distributions of Clonazepam (Clon) and 7-Aminoclonazepam (7AC). Among Clon and/or 7AC positive specimens, only 1,329 (56%) were positive if the analyte Clon alone was used to detect Clon use, leaving a significant percentage of specimens not identified for Clon use. In contrast, 2,207 (92%) were positive if 7AC was used. Additionally, in cases where both the Clon and 7AC were present, the metabolite was generally present in higher concentrations than the parent compound. The data from this analysis of OF sample results support the recommendation that testing for both 7AC and Clon enhances sensitivity for detecting Clon use compared to testing for Clon alone.

Conflict of Interest

Authors are current employees of Quest Diagnostics & have received salaries and may own stock.

P-067

Long-term Stability of Isotonitazene and Protonitazene in Whole Blood

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Abstract

Introduction: Storage of specimens potentially containing forensically relevant drugs is an important consideration for determining laboratory workflows and standard operating procedures. Analyte stability can be affected by environmental conditions (e.g., temperature) or sample-specific factors (e.g., bacteria or enzymatic activity). Although these factors are typically mitigated with preservatives and temperature control, there may be times samples are subjected to varying conditions, such as temperature changes, during periods of power loss, transport, or on the benchtop during sample preparation. Long-term stability is also an important consideration as some case samples may need to be tested or re-tested weeks or months after initial receipt. With the continual emergence of new compounds, such as nitazene analogs, assessing the stability of each individual analyte over extended periods presents a significant challenge. Therefore, the identification of degradation products may indicate analyte loss if samples have been subjected to lengthy storage times. As nitazenes continue to proliferate in the United States novel opioid market, research regarding their stability under varying conditions can help inform best storage practices for suspected nitazene samples.

Objectives: This study sought to qualitatively evaluate the stability of isotonitazene and protonitazene over a six-month period under five storage conditions (frozen, -20°C; refrigerated, 4°C; ambient, ~20°C; elevated, 35°C; and freeze/thaw cycles) in whole blood.

Methods: Whole, preserved (1% sodium fluoride, 0.2% potassium oxalate (w/v)) bovine blood samples were prepared in bulk at two target concentrations (10 ng/mL and 50 ng/mL), aliquoted into red-top blood tubes, and placed in their respective storage locations for a total of six months. Samples were prepared with a simple liquid-liquid extraction utilizing ammonium hydroxide, borate buffer, and 1-chlorobutane and were subsequently analyzed with a validated qualitative LC-QTOF/MS method. At designated intervals (daily for one week, weekly for one month, and monthly for six months), duplicate aliquots (n=2) were taken from each storage condition and processed according to the validated workflow. Relative peak area ratios (RPA) were compared to those of the initial time point (t^0) to determine stability, with RPAs greater than ±20% of t^0 for two consecutive time points deemed unstable. Data were also monitored throughout the study for the presence of any potential degradation products.

Results: Both analytes demonstrated stability for the entirety of the study across conditions at both concentrations, except for freeze/thaw conditions, which were acceptable for nine of 12 cycles. Presumptive identification of the degradation products *N*-desethyl isotonitazene and *N*-desethyl protonitazene occurred as early as 14 days under elevated conditions, at three weeks in frozen samples, and four weeks in ambient samples, all with acceptable chromatographic performance. Under refrigeration (4°C), degradation products were only observed in the final testing time point (6 months) for high concentration samples (50 ng/mL).

Discussion: Evaluating the stability of emerging drugs is important for informing storage protocols in forensic toxicology laboratories but can be challenging to assess for novel substances. This study evaluated the six-month storage stability of two forensically relevant

nitazene analogs. While the analytes remained stable under most conditions during the monitoring period, the identification of *N*-desethyl isotonitazene and *N*-desethyl protonitazene as degradation products in all conditions during at least one time point could complicate toxicological interpretation since these are the primary metabolites of isotonitazene and protonitazene, respectively. Identification of degradants in refrigerated samples at only the final time point suggests this may be the most suitable storage condition for samples in suspected nitazene cases. Laboratories should be aware of this potential degradation when evaluating nitazene samples, especially those at high concentrations, to prevent misinterpretation of analytical findings. Future studies using a quantitative LC-MS/MS method to further characterize the degree of degradation will be explored.

P-068

Cross-Reactivity of 53 Cannabinoid Analogs and Metabolites in Urine Using Enzyme-Linked Immunosorbent Assay (ELISA) and Homogenous Enzyme Immunoassay (HEIA) Carboxylic Acid and Immunalysis Synthetic Cannabinoid Kits

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Abstract

Introduction: The cannabinoid market has experienced exponential growth with the emergence of novel synthetic and semi-synthetic cannabinoid analogs. Structural differences from D9-THC are found at the position of the cyclohexenyl bond in the alicyclic ring, acetylation of the hydroxyl on the aromatic ring, or alteration of the alkyl chain length. The primary D9-THC metabolite, 11-nor-9-carboxy-THC (D9-THC-COOH), is commonly used to presumptively assess marijuana consumption and major cannabimimetic series also have commercially available immunoassay kits. Emerging synthetic and semi-synthetic cannabinoids may cross-react with commercially available cannabinoid/THC immunoassays due to structural similarities in both the parent compounds and their respective metabolite molecules.

The cross-reactivity of 27 cannabinoid analogs and 26 commercially available metabolites were evaluated on the Medica EasyRA Enzymatic Immunoassay analyzer with the Immunalysis Cannabinoids (cTHC) and Synthetic Cannabinoids 1-3 homogenous enzyme immunoassay (HEIA) kits and the OraSure Technologies Cannabinoids Intercept Microplate EIA on the Dynex DSX Automated Enzyme-Linked Immunosorbent Assay (ELISA) system at the D9-THC-COOH cut-off of 50 ng/mL and the synthetic cannabinoid kits at the 10 ng/mL cutoff. Cross-reactivity was evaluated at concentrations of 20, 50, 100 and 1,000 ng/mL in urine in triplicate.

Objectives: To define the impact of the analogs and analog metabolites on current urine drug screening immunoassay methods and understand the relevant structural moieties contributing to cross-reactivity, and the consequential challenges and application.

Methods: All analogs were prepared in verified cannabinoid free urine. ELISA verification controls were prepared relative to the cutoff at 2X, 1X, 1/2X, along with a positive (200 ng/mL D9-THC-COOH) and negative quality control (blank urine). For the HEIA Cannabinoids cTHC kit, calibration was performed at 50 ng/mL for Δ9-THCCOOH, with controls set at ±25% of the cutoff. The synthetic cannabinoid kits were calibrated at 10 ng/mL to the relevant N-pentanoic acid metabolite (JWH-018, UR-144 or AB-PINACA), with controls at ±50% of the cutoff, as specified by Immunalysis. HEIA uses glucose-6-phosphate dehydrogenase (G6PDH) to convert NAD to NADH and an absorbance measurement is read at 340 nm. If cross-reactivity was observed at 1000 ng/mL, subsequent dilutions were prepared and reanalyzed. Compounds that generated a negative result at 1000 ng/mL were considered undetectable.

Results: For the D9-THC-COOH calibrated kits, five parent THC analogs at 1000 ng/mL (19%) and two at 500 ng/mL (7%) were positive at the 50 ng/mL cutoff concentration on the ELISA platform. Twelve parent analogs at 1000 ng/mL (44%), four at 500 ng/mL (15%), and one at 100

ng/mL (3%) were positive at the 50 ng/mL cutoff concentration on the HEIA platform. One THC analog metabolite at 1000 ng/mL (4%), five at 500 ng/mL (19%), nine at 100 ng/mL (35%), and 2 at 50 ng/mL (8%) tested positive at the 50 ng/mL cutoff concentration on ELISA. Two THC analog metabolite at 1000 ng/mL (8%), three at 500 ng/mL (12%), and thirteen at 100 ng/mL (54%) tested positive at the 50 ng/mL cutoff concentration on HEIA. All analogs were negative at 1000 ng/mL for the three synthetic cannabinoid analog kits.

Discussion: The results on both instruments indicate cross-reactivity at possible relevant concentrations for the carboxy and hydroxy metabolites when calibrated to D9-THC-COOH. The number and position of bonds in the alicyclic ring, the complete cyclic ring compared to the broken cyclic ring as in CBD, and the carboxylic acid group on the alicyclic ring significantly contributed to cross-reactivity. The length of the alkyl sidechain impacted cross-reactivity minimally. The results indicate that high presumptively positive THC rates for delta-9-THC-COOH could be due to the cross-reactivity with cannabinoid analog metabolites and demonstrate certain moieties permit cross-reactivity.

Conflict of Interest

This project was funded by the National Institutes of Justice (15PNIJ-23-GG-01421-COAP) and the National Institute of Health (NIH-P30-DA033934).

P-069

The Benefits of Centrifugation on Oral Fluid Analysis: Examining Volume and Analyte Recovery

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Abstract

Introduction: Oral fluid is becoming an increasingly popular matrix due to its ease of collection. While oral fluid is a relatively simple matrix to collect, the collection kits can cause issues downstream. Most kits consist of an applicator with attached sponge, a tube filled with 3 mL of buffer solution, and a cap. The sponge is used to collect 1 mL of oral fluid and is then placed in the buffer solution to ensure analyte stability and inhibit bacterial growth. The total volume of the solution for testing should be 4 mL (1 mL of oral fluid and 3 mL of buffer), however, it is often very difficult to recover the full 4 mL of solution. There are different techniques to manipulate the sponge and improve sample recovery. Some popular techniques include manual compression and centrifugation. The goal is to remove the full volume of liquid from the sponge to improve analyte recovery. In this work, these two techniques will be compared by examining volume recovery and analyte recovery.

Objectives: The primary objective of this work is to demonstrate the advantages of incorporating a centrifugation step to sample preparation, and its impacts on volume and analyte recovery when performing analysis of drugs of abuse and (DoA) in oral fluids by LC-MS/MS.

Methods: An LC-MS/MS method was developed using a biphenyl analytical column. Mobile phase A consisted of 0.1% formic acid in water and mobile phase B consisted of 0.1% formic acid in methanol. A total of 31 commonly abused drugs were separated under gradient conditions, with a total cycle time of 7 minutes. Samples were prepared in synthetic oral fluid and combined with Quantisal™ buffer. Two sample recovery techniques were compared and tested in triplicate: manual compression and centrifugation. Samples were prepared using a salt-assisted liquid-liquid extraction (SALLE) with a saturated sodium chloride solutions. Samples were then dried down under nitrogen and reconstituted in 90:10 mobile phase A: mobile phase B, before moving to the instrument for analysis.

Results: The two techniques were compared 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 using each technique. When using centrifugation, on average, an additional 200 µL were collected compared to the manual compression. Analyte recovery was compared by spiking a known concentration, 50 ng/mL, into each of the samples. These samples were evaluated using a calibration curve prepared by only using 1 mL of fortified synthetic oral fluid and 3 mL of buffer. The recovery and peak area of all analytes improved when using the centrifuge. Accuracy and precision of the techniques were also compared. At ±15% of target value, only 4 analytes fell withing this range when using the manual compression technique, compared to centrifugation which had 21 analytes within range. This improved to 25 analytes when the acceptance criteria was increased to ±20% of the target where the manual compression was only at 12. When assessing precision, the centrifugation technique yielded lower %RSD values for 24 analytes as compared to manual compression. The use of extraction aides was also investigated and compared to these accuracy and precision results.

Discussion: In this work, two different techniques were compared to remove liquid from the sponge of the oral fluid collection kits. Through examining volume recovery and analyte recovery, it was clear that the centrifugation technique showed the best results for total volume recovered as well as increased analyte recovery when analyzed quantitatively. Centrifugation can add time to the method, but this work highlights the necessity of centrifugation when analyzing DoA in oral fluid.

Conflict of Interest

2) Salary/Consultant

P-070

Rapid Differentiation of Δ^9-Tetrahydrocannabinol and Δ^8-Tetrahydrocannabinol in Oral Fluid Samples Using LC-MS/MS and Silver Ion Complexes

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Abstract

Introduction: Δ^9-Tetrahydrocannabinol (Δ^9-THC) is the primary psychoactive constituent of cannabis and is subject to federal regulation. In contrast, Δ^8-THC, a structural isomer, has gained popularity in commercial products due to its ambiguous legal status and perceived milder psychoactivity. Despite their chemical similarity, accurate differentiation is critical for regulatory, forensic, and clinical applications. Historically, most liquid chromatography-tandem mass spectrometry (LC-MS/MS) methods differentiate Δ^9-THC and Δ^8-THC based on variability in chromatographic retention which generally requires lengthy run times.

Objectives: To develop and validate a rapid analytical method for the reliable differentiation and quantification of Δ^9-THC and Δ^8-THC in authentic oral fluid samples. Leveraging variable binding affinities of Δ^9-THC and Δ^8-THC to silver ions which induce exclusive ion fragmentation patterns.

Methods: LC-MS/MS optimization was performed to differentiate Δ^9-THC and Δ^8-THC based on variability in ion fragmentation patterns and ion ratios of the [M+Ag] species. Authentic standards were used to establish method specificity. Silver ions were introduced using methanolic silver acetate post-column addition. Oral fluid samples (n=50) that had previously confirmed positive for Δ^9-THC or Δ^8-THC on a validated ELISA-SPE-LC-MS/MS method were analyzed.

Results: LC-MS/MS analyses of [Δ^9-THC+AG] and [Δ^8-THC+AG] with unique ion transition ratios achieved accurate assignment in differentiating between Δ^9-THC and Δ^8-THC (1 ng/ml – 500 ng/ml) utilizing a 1.3-minute method (in contrast to the previous 4-minute method used in-house).

Discussion: The analyses of [THC+Ag] species can be performed simultaneously to [THC+H] species. The [THC+H] is analyzed quantitatively for the total sum Δ^9-THC and Δ^8-THC concentration. The analysis of [THC+Ag] in this method is used as a qualitative ion that appropriately assigns “pure” samples of either Δ^9-THC or Δ^8-THC. Mixed samples of Δ^9-THC and Δ^8-THC fall out of the established ion ratios; while the total concentration remains accurately reportable, individual quantitation’s of Δ^9-THC and Δ^8-THC still require chromatographic resolution.

Conclusions

Robust analytical methods can reliably and rapidly distinguish Δ^9-THC from Δ^8-THC in oral fluid. These tools are essential for accurate toxicological interpretation and regulatory enforcement in the evolving cannabinoid marketplace in a time appropriate manner.

Conflict of Interest

Salaried employee of Forensic Fluids Labs

P-071

GHB Detection in a Drug-Facilitated Crime – A Case Report

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Abstract

Introduction: The detection of drugs can be challenging in drug-facilitated crime (DFC) cases. Depending on the type of drugs used in a DFC, there can be time limitations on the collection of a urine sample that affect the ability to detect certain drugs due to the rate of metabolism in the human body. One drug of interest in relation to DFC cases is gamma-hydroxybutyrate (GHB). GHB is a Schedule I central nervous system depressant which can cause drowsiness, confusion, memory impairment, and unconsciousness. However, GHB is metabolized very quickly and the detection window in a urine sample is approximately 12 hours from the time of administration. GHB has also been used for potential performance-enhancing purposes, like building muscle and burning fat, and as a prescription treatment for narcolepsy. In addition, gamma butyrolactone (GBL) and 1,4-butanediol (BD) endogenously metabolize to GHB and can cause similar symptoms. These compounds are found in a variety of commercially available products, including industrial solvents and cleaning solutions. GHB is also naturally produced in the body in very small amounts and is a precursor to other naturally occurring compounds in the brain.

Objectives: To share a suspected drug-facilitated crime case in which GHB was qualitatively confirmed in the victim's urine.

Methods: The urine sample was prepared using liquid-liquid extraction in ethyl acetate in an acidic environment. The extracts were derivatized with BSTFA (with 1% TMCS). Duplicate extractions were completed for confirmation, using 1mL of urine per extraction. Two positive controls (10µg/mL and 25µg/mL) and one negative control were extracted alongside each replicate. The samples were analyzed on Agilent 7890A Gas Chromatograph/5975C Mass Spectrometer on selected-ion monitoring (SIM) mode, monitoring ions 117, 204, and 233. Data was analyzed using Agilent MSD ChemStation (F.01.00.1903).

Results: In the case presented here, a urine sample from an individual involved in a suspected DFC was collected approximately 7 hours after the incident and analyzed, qualitatively confirming the presence of GHB; no other drugs or intoxicants were able to be confirmed.

Discussion: The illicit use of GHB is one of many ways a DFC can occur, however it is not identified in urine frequently. Often, the victim does not report right away for a variety of reasons, so the medical examination and urine sample collection may occur well outside the estimated window of detection. In this case, the victim was given a drink and candy by another individual and reported that they did have any memory after that until the following morning. After waking, the victim reported feeling "very high" and "out of control" of their body and had vomited and lost control of their bowels at some point prior to waking. They were taken to the hospital by medical personnel, where a sexual assault forensic exam (SAFE) kit was collected, including a urine sample. The symptoms reported by the victim are consistent with symptoms observed with central nervous system depressant impairment. In a survey of GHB intoxication [SS2] cases conducted by Kapitány-Fövény et al. (2017), approximately half of the individuals involved in alleged DFCs reported having blackouts and a quarter of the individuals reported memory loss.

P-072

Comprehensive Drug Analysis in Hair Samples: Extraction & UHPLC-MS/MS Quantification

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Abstract

Introduction: The use of hair as a biological matrix for forensic testing continues to increase in popularity. As a specimen, hair offers several benefits. In contrast to other matrices such as blood and urine, hair collection is simple and does not require medically trained staff to collect the sample. Sample collection is not considered intrusive, enabling easy supervision, thus reducing the potential for sample adulteration. Furthermore, once collected, hair can be easily transported and stored at room temperature prior to analysis. One key benefit of hair is that it provides an extended window of detection for drug exposure. Unlike traditional matrices such as blood and urine, where drugs may only be detected within hours or days of use, drugs can be detected in hair months and even years after use. This makes analysis of illicit drugs and pharmaceuticals in hair useful for situations where other samples may not be appropriate or available for analysis such as determining drug exposure in post-mortem toxicology, drug facilitated sexual assault (DFSA), or for other forensic testing in which long-term monitoring is desired.

Objectives: This study aimed to develop, optimize and validate a method for the extraction and quantification of a comprehensive panel of drugs in hair to satisfy the confirmation cut-off values recommended by the Society of Hair Testing (SoHT)[1].

Methods: Hair samples were decontaminated by sequential washing with acetate buffer, methanol, acetonitrile and acetone. They were then pulverized, weighed into vials (20 ±1 mg), and incubated in a proprietary solvent mixture for 2 hrs at 95 °C. They were then extracted by mixed-mode cation exchange plates (Waters Oasis™ MCX) and analyzed by UHPLC-MS/MS (Waters Xevo™ TQ-Absolute). The drug panel included opiates and synthetic opioids (*N*=20), amine stimulants (*N*=5), benzodiazepines (*N*=20), and other common drugs of abuse such as cocaine, PCP and methylphenidate (*N*=11). Sample preparation conditions were adjusted for optimal extraction of all compounds, including hair pretreatment, initial incubation conditions and solid phase extraction.

Results: Efficient and clean sample extraction enabled a robust method that was validated for linearity, sensitivity, accuracy and precision for a total of 56 analytes according to ASB 036. Extraction recoveries ranged from 6-79% with %CVs <17% for all analytes. Greater than 70% had recoveries exceeding 50%. Matrix effects (ME) ranged from 0-60% with all S.D. values <10%. Greater than 70% had ME <30%. The chromatographic method was 4 minutes, with all peaks eluting by 3.1 minutes while achieving baseline separation from potential interfering isomers. Limits of quantification, established by sequential dilutions reached as low as 0.01 ng/mg for most analytes and 0.001 ng/mg for fentanyl, enabling all analytes to meet the recommended SoHT confirmation cut-offs. Both intra and inter-batch accuracy and precision met validation requirements. All analytes in external QC samples were positively identified and greater than 80% had concentrations that fell within the manufacturer's acceptance range. All %CVs were <10% (*N*=5).

Conclusions

We have developed a procedure for the extraction and analysis of a comprehensive drug panel in hair samples that meets the requirements of SoHT for sensitivity. The sample preparation method resulted in consistent recoveries and well-controlled matrix effects. Quantitative results were accurate and precise over the entire calibration range. The combination of efficient sample preparation, rapid chromatography and accurate and precise quantification resulted in a method that can be used to accurately quantitate a variety of drugs in hair samples.

For Forensic Toxicology Use Only.

[1]-Society of Hair Testing Guidelines for drug testing in hair. *Forensic Sci. Int.* 2012. 218, 20-24.

Conflict of Interest

I am a full-time, salaried employee of Waters Corporation.

P-073

Post-Mortem Drug Screening of 364 Drugs Using a Combination of LC-QQQ and LC-QTOF with Positive and Negative mode Electrospray Ionization

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Abstract

Introduction: Quadrupole Time of Flight (QTOF) and Triple Quadrupole Mass Spectrometry (QQQ) combined with Liquid Chromatography (LC) have emerged as valuable drug screening techniques in the toxicology laboratory. LC-QTOF and LC-QQQ offer several key advantages over traditional drug screening approaches (i.e. immunoassays), particularly in terms of specificity and scope. With LC-QTOF, the high mass accuracy and resolving power combined with the ability to perform tandem MS greatly improves confidence in drug identification. Additionally, QTOF data can be analysed retrospectively, particularly when operated in a data independent fashion; a powerful feature given the ever-changing landscape of post-mortem forensic toxicology and constant emergence of Novel Psychoactive Substances (NPS). A challenge for LC-QTOF based screening is sensitivity. Potent opioids, which require detection limits in the pg/ml range, often require extensive sample cleanup and a concentration step to achieve adequate limits of detection by LC-QTOF. LC-QQQ is an excellent complimentary screening method as pg/ml detection limits are achievable without rigorous sample preparation.

Objectives: The target of this work was to develop and validate a drug screening method that combines a low volume, simple, and rapid sample preparation with LC-QQQ and LC-QTOF analysis. A specific and unique focus of the QTOF based screening was to include negative mode Electrospray Ionization (ESI). Certain classes of drugs (e.g. barbiturates, cannabinoids, acidic drugs) are not amenable to positive mode ESI. Fast polarity switching can provide simultaneous positive and negative mode acquisition but is challenging on the instruments electronics and is generally not recommended. Therefore, acquisition in both ESI modes typically requires duplicate injections which in turn leads to longer acquisition times and reduced sample throughput. Here we present a LC-QTOF screening method which utilizes a dual pump and LC column setup to perform positive and negative ESI mode analysis with minimal reduction in efficiency. LC-QQQ analysis was also performed to achieve pg/ml detection limits.

Methods: Sample preparation consisted of a protein precipitation using either 50 or 100 µl of post-mortem blood. The extract was split and simultaneously analysed by LC-QTOF and LC-QQQ. For LC-QTOF analysis, each sample was injected twice, once for positive ESI acquisition and once for negative mode ESI acquisition. A dual pump system was used to improve sample throughput; a quaternary pump was used to rinse and equilibrate one column while a second column is being used for the analytical run. A low energy spectrum (0 V) was collected for determination of the intact or parent compound, and a high energy spectrum (20 V) for identification of characteristic fragment ions.

Results: An LC-QTOF and LC-QQQ based screening method was developed and validated to routinely screen for 364 compounds. Limit of detections were determined for all compounds during method validation, which also included interference and processed sample stability. The method has been applied in routine use and has screened thousands of post-mortem

blood samples in addition to urine and liver homogenates. The methods applicability was also demonstrated in the analysis of the lab's proficiency test samples.

Discussion: The ability to screen for drugs in both positive and negative mode ESI on an LC-QTOF system provides a very robust, specific, and flexible drug screening approach. The addition of negative mode ESI analysis was critical to include barbiturates, 11-Nor-9-carboxy THC, valproic acid and several other drugs to the screening panel. For potent drugs (e.g. carfentanil), targeted LC-QQQ analysis allows for limits of detection in the mid pg/ml range. The methods validity and applicability has been demonstrated in extensive routine casework analysis and proficiency test samples.

P-074

Prevalence of Stimulant Drugs and Benzodiazepines in Sexual Assault Casework in Dallas County from 2018 - 2023

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Abstract

Introduction: The Dallas County Southwestern Institute of Forensic Sciences (SWIFS) has a toxicological workflow specifically for sexual assault cases, including the analysis of blood and urine when collected in a case, giving SWIFS the unique ability to compare results between the specimen types. Specimens are also tested regardless of collection time relative to the time of assault. Due to the prevalence of stimulant drugs in DFSA casework, the Laboratory wanted to evaluate positivity rates in blood and urine specimens from suspected sexual assault cases in Dallas County. Though not as frequently reported in DFSA casework, benzodiazepines were also investigated due to their sedating effects and historical positivity.

Objectives: To assess the prevalence of stimulant drugs and benzodiazepines in sexual assault casework in Dallas County and evaluate congruency between blood and urine toxicological results related to these drugs.

Methods: Sexual assault case samples submitted to the Toxicology Laboratory at SWIFS between January 1, 2018, and December 31, 2023, were reviewed for positive stimulant drugs and benzodiazepines results. In cases in which blood and urine specimens were received and analyzed, toxicological results were evaluated for consistency between the two matrices.

Results: Over the 6-year period, SWIFS received 1,835 requests for analysis in sexual assault cases, accounting for 3.7% of the casework. Of those 1,835 requests, 1,297 (70.6%) were confirmed to have a positive drug result. Of those 1,297 requests, 684 (52.7%) were positive for a stimulant drug and/or benzodiazepine. In this subset of cases, 436 were submitted with blood and urine specimens.

The most prevalent analytes in both matrices were benzoylecgonine and methamphetamine. Benzoylecgonine was confirmed in 130 of the blood samples, whereas it was confirmed in 300 of the urine samples. Similarly, methamphetamine was reported in 125 of the blood samples and 301 of the urine samples. The most prevalent benzodiazepine in both matrices was alprazolam, reported 35 times in blood and 55 times in urine. Quantitative results were only reported in blood specimens and are summarized below (Table 1).

Table 1. Stimulant drugs and benzodiazepines in blood and urine, regardless of paired submission.

		Blood					Urine
		LOD/LOQ (ng/mL)	Count	Minimum (ng/mL)	Maximum (ng/mL)	Average (ng/mL)	Count
Benzodiazepines	clonazepam	5	14	10.0	46.0	24.5	7
	7-aminoclonazepam	5	20	10.0	81.0	31.2	39
	alprazolam	5	35	10.0	291.3	56.7	55
	α-hydroxyalprazolam	5	1		Reported Qualitatively		38
	diazepam	5	18	7.2	189.0	41.5	2
	demethyldiazepam	5	25	5.4	167.0	36.0	12
	temazepam	5	1		12.0		10
	oxazepam	5	0				4
	lorazepam	5	15	6.0	49.0	19.2	8
	midazolam	5	1		67.8		0
Stimulant Drugs	methamphetamine	10	125	10.1	1417.4	134.3	301
	amphetamine	10	88	10.6	340.0	48.4	296
	cocaine	10	33	10.4	124.5	26.6	187
	benzoylecgonine	50	130	50.1	4388.2	494.4	300
	cocaethylene	10	15	10.5	95.7	32.0	75
	ecgonine methyl ester	10	76	10.4	522.4	54.8	300
	MDA	10	6	11.6	30.6	95.1	12
	MDMA	10	8	33.1	278.9	175.3	14

MDA – methylenedioxyamphetamine; MDMA – methylenedioxymethamphetamine

Unsurprisingly, the analytes were reported more frequently in urine specimens, with the exception of diazepam, demethyldiazepam, and lorazepam. A further evaluation of cases which contained paired blood and urine samples revealed that 88% of the urines were positive for a stimulant whenever the blood was also positive. Conversely, if a urine sample was positive, positivity rate in the corresponding blood sample ranged from 19.6-61.5% for stimulant drugs. Congruency between benzodiazepines blood and urine results was more variable.

Discussion: Stimulant drugs were more likely to be found in both specimens, whereas benzodiazepines were more variable. The extensive metabolism and longer half-lives associated with benzodiazepines may explain why certain drugs in this class had higher positivity in one matrix over the other. The data supports urine as the preferred matrix in DFSA casework as urine allows for a longer window of detection of drugs. However, paired results in blood can be valuable in regard to interpretation of potential impairment of the individual.

One limitation of this data set is information related to time of alleged incident and time of collection is unknown, which impact the detection and interpretation of drugs in these cases.

P-075

Long-Term Stability of Tricyclic Antidepressants in Human Urine

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Abstract

Introduction: Urine collection is a method used in clinical and forensic toxicology. Tricyclic Antidepressants (referred to as TCAs hereafter) are used to treat conditions like major depressive disorder, anxiety disorders, and certain types of chronic pain. A presumptive positive identification of a tricyclic antidepressant in urine using a TCA antibody enzyme immunoassay screening by a Chemistry Analyzer, enzyme-linked immunosorbent assays, or lateral flow membrane tests requires shipping to a laboratory for confirmation testing.

TCAs' stability in urine cup samples is not well characterized; therefore, the study is required to characterize the stability of TCA analyte compounds (Nortriptyline, Amitriptyline, Desipramine, Desmethyldoxepin, Doxepin, and Imipramine) under ambient, refrigerated, and frozen conditions, in support of the design history file for the SureStep™ TCA One Step Tricyclic Antidepressants Drug Screen Test Strip/Device, and characterizes the effects of temperature on the analytes.

Objectives: The attendees will learn about the stability assessment protocol developed by Abbott Redwood Toxicology Lab for TCAs in urine, including the design of validation, planning of tests, preparation of contrived specimens, and evaluation of analyte stability by SWGTOX guidelines, as well as the subsequent validation report findings.

Methods: A protocol was developed to assess the stability of TCAs in urine using LC-MS/MS, with modifications from a single-point calibration qualitative method to an ASB standard 036 recommendation.

Methanol stock standards (1 mg/mL) were used to prepare a six-calibrator set (25, 50, 100, 500, 1000, and 2500 ng/mL) in human urine (UTAK) diluted 1:1 in deionized (DI) water with 0.1% preservative.

Preliminary QC batch testing confirmed the analytical measurement range of 25-2500 ng/mL, with six replicates for each calibrator. A quality control set of test solutions was prepared at concentrations of 50 and 500 ng/mL. Human urine (UTAK) was thawed, its pH was checked, and it was fortified with TCAs to create test solutions. To ensure homogeneity, the solutions were mixed 30 times by inversion and then left to stand overnight in the refrigerator. Two single 100 mL solutions were divided into six 90 mL sample cups per time point.

An internal standard solution of 200 ng/mL Amitriptyline-D3 in acetonitrile is prepared, bottled, and frozen for use at all time points.

Urine samples are extracted with acetonitrile and purified by centrifugation. Separation was performed using Shimadzu Prominence LCs and Sciex 3200 MS/MS systems (with a Phenomenex Phenyl Hexyl column). Three replicate samples per concentration were analyzed at 0, 14, 30, 60, 90, 180, and 360 days under ambient (20°C), refrigerated (4°C), and frozen (-20°C) conditions. Each analyte was monitored using two MRM transitions and one for the internal standard in positive mode.

Results: Temperature was monitored at 1-minute intervals using Vaisala RF100 data loggers to ensure that temperature ranges were within specification. Regression analysis using Minitab statistical software predicts the analyte shelf life of more than 360 days for refrigerated and frozen samples. The analyzed room temperature (20°C) specimen results had shelf lives as follows:

- Amitriptyline 40 days
- Desipramine 40 days
- Desmethyldoxepin 225 days
- Doxepin 136 days
- Imipramine 83 days
- Nortriptyline 242 days

Discussion: The data ensures the pre-analytical stability of the TCA drugs and profiles the slow degradation of the analyte material under ambient conditions, where refrigerated and frozen samples maintain the integrity of the parent drug concentration.

Conflict of Interest

Salary and Stock in Abbott Laboratories

P-076

Oral Fluid Collection Device Stability Assessment of Twenty-Two Analytes in Human Saliva

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Abstract

Introduction: The stability of drugs of abuse in oral fluid post-collection is critical for accurate toxicological analysis and reliable detection over extended storage periods. Oral fluid collection is a non-invasive method used in criminal justice, family courts, and DUI programs. This study assesses the stability of drug analytes in oral fluid stored in Quantisal buffer under refrigerated and frozen conditions. This study supports Abbott Toxicology's storage protocols and ensures the reliability of results in forensic and clinical settings.

Objectives: This study outlines the stability assessment protocol developed by the Abbott Redwood Toxicology Lab, including the design of validation, planning of tests, preparation of specimens, and evaluation of analyte stability in accordance with SWGTOX guidelines.

Methods: A protocol was developed to assess the stability of opiates, amphetamines, cocaine, cannabinoids, phencyclidine, and fentanyls in oral fluid using LC-MS/MS.

Methanol stock standards (1 mg/mL) were diluted to achieve 100 times the target low and high concentrations. Human saliva (GoldenWest®) was thawed, centrifuged, and fortified with 1% methanol standards. To ensure robustness and reproducibility, three lots of Quantisal devices were used to collect 1 mL of fortified saliva via dipped cellulose pads, which were then stored in 3 mL of buffer, incubated for 18–24 hours, and subsequently filtered and aliquoted. Six devices per concentration were prepared at each time point to ensure a sufficient volume, with three replicates per device analyzed per method.

THC, opiates, and PCP were extracted via liquid-liquid extraction; cocaine/BE, amphetamines, and fentanyls used dilute-and-shoot methods. Separation was performed using Shimadzu Prominence LCs and Sciex 3200/4000 MS/MS systems across six validated methods (five using Phenomenex® Phenyl Hexyl columns, THC using Supelco® C18). Nine samples per concentration were analyzed at 0, 30, 60, and 90 days under refrigerated (4°C) and frozen (-20°C) conditions. Each analyte was monitored using two MRM transitions and one for the internal standard in positive mode.

Results: Temperature was continuously monitored using Vaisala RF100 data loggers. All analytes met U.S. toxicology stability criteria for up to 90 days under both storage conditions, being within a range of 40% to 140% of the day 0 mean value. Refrigerated samples exhibited minor degradation in some analytes, whereas frozen samples maintained analyte integrity more consistently. Most analytes remained within ±10% bias from baseline. Notably, cocaine showed conversion to benzoylecgonine, and 6-acetylmorphine showed conversion to morphine under refrigerated conditions.

Discussion: The data confirms the pre-analytical drug and drug metabolite stability for opiates (morphine, oxymorphone, hydromorphone, codeine, oxycodone, hydrocodone, and 6-acetylmorphine), amphetamines (amphetamine, methamphetamine, MDEA, MDMA, and MDEA),

cocaine (cocaine, benzoylecgonine), cannabinoids (Delta-8-THC, Delta-9-THC), phencyclidine, and Fentanyls (Fentanyl, Acetyl Fentanyl, Butyryl Fentanyl, Furanyl Fentanyl, and Acryl Fentanyl) and profiles the slow conversion of 6-acetylmorphine to morphine and cocaine to benzoylecgonine under refrigerated conditions, where frozen samples maintain parent drug concentration integrity. We will present regression analysis using Minitab® statistical software to predict analyte shelf life beyond 90.

Conflict of Interest

Salary and Stock in Abbott Laboratories

P-077

Observed Prevalence of Delta-8 and Delta-9-carboxy-THC by Age in the Kansas City Area

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Abstract

Introduction: Delta-9-tetrahydrocannabinol (Δ-9 THC) is the primary psychoactive component of the cannabis plant (Cannabis sativa). Δ-8 THC, an isomer of Δ-9 THC differing only in the position of one double bond, is also a naturally occurring psychoactive cannabinoid with psychotropic effects similar to Δ-9 THC. Because it is present in significantly lower concentrations than Δ-9 THC in natural preparations, Δ-8 THC is generally synthesized from cannabidiol (CBD). Missouri legalized recreational cannabis for adults aged 21 and older in December 2022, but at that time Missouri was among the list of states that did not have age restrictions for Δ-8 THC product sales. An executive order banning the sale of food and drink products containing unregulated psychoactive cannabis products, including Δ-8 THC. went into effect September 2024. Though the market and laws continue to evolve, there is limited research specific to Δ-8 THC usage in adult and pediatric populations. Both Δ-8 THC and Δ-9 THC are commonly detected as their carboxylic acid metabolite (Carboxy-THC).

Objectives: To examine the prevalence of Δ-8 and Δ-9-carboxy-tetrahydrocannabinol (Carboxy-THC) positivity within different age groups in the Kansas City area.

Methods: Children's Mercy Hospital screened 10,278 urine and blood samples from January 2023 through December 2024—both clinical and forensic—for carboxy-tetrahydrocannabinol (THC) metabolite using an enzyme-multiplied immunoassay technique (EMIT). Samples that screened positive by immunoassay were quantitatively confirmed using GC/MS, with a limit of quantitation (LOQ) of 15 ng/mL.

Results: Of the 10,278 samples that screened positive for cannabinoids by immunoassay, 2,146 (21%) were confirmed positive for Δ-8 and/or Δ-9-carboxy-THC. The data were stratified by age groups, as shown in the table below. A significant number of samples within all age groups confirmed the presence of both Δ-8 and Δ-9-carboxy-THC. Prevalence of Δ-8-carboxy-THC increased with age, peaking in the 13–18-year age group, followed by a notable decline after age 18. Similarly, Δ-9-carboxy-THC positivity rose significantly after age 13; however, unlike Δ-8, its prevalence remained high beyond age 18. At the time of presentation, the data will be further broken down by percentage positivity across age, gender, sample type (blood versus urine), and clinical versus forensic categories.

Discussion: Overall, the prevalence of THC usage in the Kansas City area is significant with 21% of all screened samples confirming the presence of THC as its carboxylic acid metabolite. THC usage rises markedly after age 13. The prevalence of Δ-8 THC use is notable, and concurrent use of both Δ-8 THC and Δ-9 THC is commonly observed.

10278 Samples Screened by EMIT for Cannabinoids from 2023-2024, Positivity by GC/MS Confirmation:

Age (years)	**Δ-8 Only Positive**	**Δ-9 Only Positive**	**Both Δ-8 and Δ-9 Positive**	**Total Positive**
0-5	14	79	54	147
6 to 12	10	37	27	74
13 to 18	149	495	705	1349
>18	1	417	158	576
Total Positive, All Ages	174	1028	944	2146
% Positive, All Ages	8 %	48 %	44 %	---
% Positive, All Samples	1.7 %	10.0 %	9.2 %	20.9 %

P-078

Method Optimization and Validation for the Detection of Single Doses of Diphenhydramine in Hair Specimens from a Controlled Dosing Study

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Abstract

Introduction: Hair is a valuable forensic toxicology matrix due to its large window of detection for drugs. It takes an average of 10 days after time of dosing for drugs to incorporate into the hair matrix and grow out of the scalp. Beyond that period, drugs can be detected weeks to months after exposure. This can be especially helpful in cases of drug facilitated sexual assault (DFSA) and drug facilitated crime (DFC), where a single dose of a drug is suspected.

Objectives: Hair analysis procedures can be extremely tedious, requiring several steps and multiple changing variables. Additionally, interpretation of the results can be complicated. A primary aim of this study was to optimize the extraction procedure and Liquid Chromatography Triple Quadrupole Mass Spectrometry (LC-QqQ-MS) quantitative method to facilitate the analysis of single dose samples. It employed a statistical Design of Experiment (DoE) approach to compare hair extraction parameters for drugs and metabolites using authentic hair reference materials (HRM). The optimized method was then fully validated according to the ANSI/ASB Standard 036 guidelines with a particular focus in achieving limits of detection (LOD) and limits of quantitation (LOQ) at levels to relevant to DFC's. Subsequent to this validation, authentic hair specimens collected from a single dose diphenhydramine (DPH) study were analyzed with the validated method with the purpose of detecting the single dose exposure.

Methods: The hair was decontaminated and homogenized into a fine powder based on previously optimized methods. The solvent swelling technique consisted of incubating the hair in a mixture of methanol, acetonitrile, and 2 mM ammonium formate (25:25:50, v/v/v) at 37ºC.

This project utilized a DoE protocol to evaluate different parameters for the extraction technique. Specifically, a 2^3 factorial assessment was used to evaluate different sample sizes (5 or 20 mg), extraction times (2 or 6 h), and the effect of an ultrasonic bath (with or without) on the method, which has been shown to improve recovery of certain drugs from hair. HRM with known concentrations was utilized to compare the extraction efficiency with each changing variable.

A controlled single dosing study was conducted with 30 individuals of varying hair color and type. Each participant was given a single dose of DPH and samples of hair were collected 30 and 60 days after dosing. All samples were put through segmental hair analysis, where hair was cut into 5 mm segments from root to tip and analyzed independently according to the optimized method. A LC-QqQ-MS method using an Agilent 1290 UHPLC coupled to a 6470 LC-QqQ-MS/MS was developed screening and quantification of 45 drugs and metabolites potentially relevant to DFC/DFSA investigation.

Results: Authentic HRM pooled from 11 different drug positive doners was previously screened and confirmed for 16 different drugs and metabolites. This homogenized sample was analyzed using the DoE studies along with four other authentic individual donor samples with a range of 2 to 9 different drugs and metabolites. Within the HRM samples, the 5 mg sample size and the 6-h extraction yielded better recovery. The highest preforming overall method consisted of a 5 mg

sample of hair with a 6-h extraction time and the inclusion of ultrasonication. For the statistical analysis within the DoE matrix, p-Values show statistical significance for the 5 mg sample size in all drugs across all HRM samples. While the percent recoveries of the other two factors showed general trends for certain drugs, extraction time and ultrasonication did not demonstrate statistical significance.

Conclusion

This research presents an optimized working method for quantitative analysis of drugs in hair. The LC-QqQ-MS method demonstrates good selectivity and sensitivity for all 45 drug compounds, suitable for the detection of low concentrations of drugs in hair consistent with single doses.

P-079

Effective Communication Can Transform a Toxic Result

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Abstract

Introduction: In data-driven and siloed environments, the act of dialogue can be undervalued. A focus on laboratory results can lead to a marginalization of scene information, autopsy findings, and clinical data. Departmentally contained toxicology labs promote interpersonal relationships that lead to better communication. This can prevent assumptions that lead to incomplete or improper specimen analysis and help tie a case together.

Objectives: Demonstrate how effective communication between the pathologist and forensic toxicologist enhances case resolution.

Methods: Four cases are highlighted where complex circumstances led to unusual toxicological results. Effective communication with pathologists about autopsy and death investigation details helped to deter incorrect conclusions concerning the cause and manner of death.

Results:

Case 1: The pathologist reported a concern for toxicity due to reported “poppers”/isobutylnitrite on scene and requested nitrite/nitrate testing. Urine nitrite test strips showed None Detected, but further testing for isobutanol was ordered by the toxicologist. The decedent had a result of isobutanol (9.3 mg/L), methamphetamine (0.20 mg/L), amphetamine (0.024 mg/L) and gabapentin (19 mg/L) in iliac blood and ethanol (70 mg/L) in vena cava blood. The pathologist indicated a plan to certify the death as methamphetamine toxicity with contributing isobutanol, gabapentin, and ethanol use. She then mentioned that the decedent had gray/black discoloration of the face and mouth. The decedent was not decomposed and last known alive the previous day. Due to the discoloration, the toxicologist ordered a methemoglobin test (78% Saturation). After consultation, the cause of death was determined to be methemoglobinemia due to isobutylnitrite toxicity with contributing ASCVD, methamphetamine, gabapentin, and ethanol use. Without active communication, the methemoglobinemia and primary contribution of isobutylnitrite to death may have been missed.

Case 2: Initial toxicology report detailed an intentional drug overdose of duloxetine, propranolol, and acetaminophen. Antemortem specimens were received from 6/14-0426 hours, 6/14-0900 hours, and 6/15 (no time noted). Testing on the specimen from 0426 hours was negative for drugs. Upon consultation with the pathologist, information emerged that the decedent exited unnoticed from the hospital after the initial blood draw, consumed medications, and was brought back to the hospital. Testing on the specimen from 0900 hours revealed acetaminophen at 220 mg/L, and cause of death is complications of acute drug toxicity including acetaminophen. Without active communication, discrepancies between the toxicology results may have led to confusion or an undetermined cause of death.

Case 3: Toxicology revealed prescribed bupropion (1.4 mg/L), hydroxy bupropion (0.59 mg/L), and propylhexedrine (present) in the iliac blood. A partner reported he would snort prescription medications and buy pills from a smoke shop. Due to the peak magnitude of propylhexedrine the toxicologist reached out to the pathologist to warn about the potential for over-the-counter inhaler

abuse. The pathologist revealed that cotton was discovered in the gastric contents during autopsy, supporting suspected inhaler abuse. Propylhexedrine was confirmed and reported present, with cause of death as toxic effects of bupropion and propylhexedrine and contributing natural disease. Active communication helped clarify the propylhexedrine oral route of administration.

Case 4: A 54-year-old female, (5'4"/190lbs/BMI:32.6) was found unresponsive and prone in bed. Although not diabetic, decedent had been using her mother's diabetes medication, Trajenta (linagliptin). Prescriptions included atenolol (not detected), paroxetine (0.32 mg/L in subclavian blood), and chlorpromazine (0.12 mg/L in femoral blood), all within count. Toxicology analysis additionally detected acetone (40 mg/dL) and isopropanol (30 mg/dL) in vitreous humor and isopropanol (29 mg/dL) in subclavian blood. In reviewing linagliptin's mechanism, discussions with the pathologist led to a hypothesis that DPP-4 inhibitors could induce a hypoglycemic event in combination with hypertension and ketoacidosis. The laboratory used a high resolution accurate mass procedure to detect a peak consistent with the hydrogen adduct of linagliptin, at *m/z* 473.2408. Linagliptin was not confirmed or reported. Due to case history and acetone/isopropanol results, the cause of death was listed as ketosis and probable hypoglycemia due to toxic effects of linagliptin.

Discussion: The pathologist statements, hospital log, autopsy findings, and reported prescription misuse/abuse all highlight the importance of clear communication between forensic partners. Onsite toxicological services can be beneficial to pathologists that may need to discuss casework complexities. Better communication leads to better outcomes.

P-080

Forensic Detection of Psychoactive Drugs in Environmental Matrices: Method Development, Degradation study, and Chiral Insights

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Abstract

Introduction: The widespread use of psychoactive pharmaceuticals such as benzodiazepines and antidepressants has resulted in their continuous release into the environment through domestic, hospital, and industrial wastewater. These compounds are often not fully metabolized and can persist in aquatic systems as parent drugs or active metabolites. While environmental scientists have investigated the ecotoxicological effects of such residues, forensic toxicology has only recently begun to consider the relevance of environmental matrices in casework. Detecting drugs in non-traditional samples such as wastewater, soil, or contaminated water sources presents both a challenge and an opportunity for forensic toxicologists. In particular, forensic reconstruction of drug use or disposal events, interpretation of background levels, and evaluation of degradation products can benefit from environmental forensic toxicology approaches.

Objectives:

This study aimed to:

1. Develop and validate a sensitive and selective analytical method for detecting and quantifying selected benzodiazepines and antidepressants in synthetic wastewater.
2. Evaluate the degradation kinetics of these compounds under simulated environmental conditions over a 14-day period, comparing biotic (microbial activity) and abiotic (sterile) environments.
3. Apply chiral LC-MS to identify and differentiate enantiomers of drugs and their metabolites.
4. Explore the forensic relevance of identifying parent drugs and their transformation products in environmental samples.

Methods: Synthetic wastewater samples were prepared in the laboratory to mimic real-world environmental matrices and were spiked with known concentrations of diazepam, etizolam, and selected antidepressants. Two parallel sets of samples were created: biotic samples containing activated microbial inoculum and abiotic controls sterilized to inhibit microbial activity. Samples were incubated at ambient temperature and analyzed at regular intervals (Day 0, 3, 7, and 14) to evaluate temporal degradation.

Sample extraction was conducted using solid-phase extraction (SPE) with two different cartridges (HLB and MCX) to compare recovery rates and optimize extraction efficiency for a range of chemical polarities. Extracts were analyzed using LC-MS, with method validation conducted in accordance with international forensic toxicology standards. Chiral analysis was performed using a specialized LC column capable of resolving the enantiomers of etizolam metabolites, fluoxetine, venlafaxine and their metabolites.

Validation parameters included selectivity, linearity ($R^2 > 0.998$), limit of detection (LOD), limit of quantification (LOQ), intra- and inter-day precision, matrix effects, and extraction recoveries.

Results: The validated LC-MS method achieved high sensitivity and specificity for all analytes, with LODs ranging from 0.2 to 0.8 ng/mL and acceptable precision (<10% RSD). Among the SPE cartridges tested, the MCX cartridge demonstrated the highest recovery for basic drugs like diazepam and etizolam, while HLB offered better selectivity for some antidepressants.

Chiral analysis allowed successful resolution of etizolam metabolite's enantiomers and the identification of its stereospecific metabolites, enabling a detailed analysis of degradation pathways. In biotic samples, significant degradation of parent drugs was observed by Day 7, accompanied by the emergence of phase I metabolites. In contrast, abiotic samples showed slower transformation, retaining higher concentrations of the parent compounds through Day 14.

The data confirmed the influence of microbial activity on drug transformation, supporting the need to distinguish between natural environmental degradation and anthropogenic alterations in forensic scenarios. The ability to detect specific degradation products and stereoisomers enhances the interpretation of forensic cases involving time-delayed sample collection, drug disposal, or environmental contamination.

Discussion: This study presents a validated method for detecting psychoactive drugs in environmental matrices, enhancing forensic intelligence in cases where biological samples are unavailable. Chiral separation supports interpretation by identifying metabolic signatures and stereoisomeric transformations. The approach broadens the evidentiary scope and highlights the value of environmental samples in modern forensic toxicology.

P-081

Screening of a Comprehensive Panel of 29 Drugs in Human Saliva Using LC-MS/MS and the Quantisal® Collection Device

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Abstract

Introduction: Saliva has emerged as a valuable alternative biological matrix for drug testing due to its non-invasive collection, reduced risk of adulteration, and ability to reflect recent drug intake. The Quantisal® oral fluid collection device standardizes sample volume and stabilizes analytes, making it suitable for routine drug monitoring. This study presents the development and validation of a robust LC-MS/MS method for the quantitative analysis of a panel of 29 drugs in human saliva collected using the Quantisal® device.

Objectives: To develop and validate a comprehensive drug testing panel comprising 29 substances—including amphetamines, opiates, benzodiazepines, cannabinoids, and select prescription medications—selected for their forensic and clinical significance, as well as their relevance to workplace drug testing requirements.

Methods: The panel included amphetamines, opiates, benzodiazepines, cannabinoids, and selected prescription medications, and were chosen based on forensic and clinical relevance their importance for workplace drug test requirements. Saliva samples were collected using the Quantisal® device, which utilizes a state-of-the-art collection technique that provides a simple, non-invasive, observed collection, avoiding issues with adulteration and dilution. Sample preparation involved protein precipitation and solid-phase extraction. Chromatographic separation was performed on a Phenomenex Kinetex biphenyl column using gradient elution with water, methanol and 0.1% formic acid as mobile phases. Detection was achieved using a triple quad LC-MS/MS system in MRM mode. Method validation followed CLIA and CAP guidelines, assessing calibration fit, sensitivity, accuracy, precision, recovery, etc. across the analytical measuring range (AMR) as shown in the table below:

Compounds	AMR (ng/mL)
6-MAM	1-1000
Alprazolam	1-1000
Amphetamine	2-2000
Benzoylecgonine	1-1000
Buprenorphine	2-2000
Carisoprodol	5-5000
Clonazepam	1-1000
Cocaine	1-1000
Codeine	3-3000
Diazepam	1-1000

Fentanyl	1-1000
Hydrocodone	3-3000
Hydromorphone	3-3000
Lorazepam	1-1000
MDMA	1-1000
Methadone	1-1000
Methamphetamine	2-2000
Midazolam	1-1000
Morphine	3-3000
Naloxone	2-2000
Nordiazepam	1-1000
Oxazepam	1-1000
Oxycodone	3-3000
Oxymorphone	3-3000
Phencyclidine	1-1000
Quetiapine	1-1000
Temazepam	1-1000
THC	2-2000
Tramadol	1-1000

Results: Six calibrators at concentrations from the LLOQ to the ULOQ were used for each analyte. Seven analytes had a linear calibration curve. The rest of the analytes used a polynomial fit to cover the relevant AMR without the need for dilution. Resulting accuracy and precision were found to be within ±20% for all analytes listed. Recoveries observed were near quantitative for most analytes, except THC and Carisoprodol which may be attributed to their lipophilicity rendering them prone adsorption. Matrix effects were measured by ion suppression mechanism using 10 spiked negative samples at two concentrations. Matrix effect was measured (1) using 10 spiked negative samples at two concentrations and found to be within ±10%. Highest matrix effects were observed in Carisoprodol at 11%, which may be due to its higher polarity and may possibly be impacted by interference from endogenous components.

Discussion: This validated LC-MS/MS method enables sensitive and specific quantification of multiple drugs in saliva using the Quantisal® device. Using a Quantisal® device offers an added advantage of ensuring adequacy of quantities in collected samples within a short time, reducing recollection and reruns and helps in shortening turnaround times for the laboratories. The method meets regulatory standards and is suitable for clinical, forensic, and workplace drug testing. The combination of Quantisal® and LC-MS/MS provides a powerful, non-invasive tool for monitoring recent drug use which is valuable information required in workplace drug tests.

Reference: (1)

1. Matuszewski BK, Constanzer ML, et al. Strategies for the assessment of matrix effect in quantitative bioanalytical methods based on HPLC-MS/MS. Analytical Chemistry. 2003;75(13):3019–3030.

P-082

When Things Have to go Fast – Screening Drug Paraphernalia using DART-HRMS

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Abstract

Introduction: Chemical analysis of the residual content of used syringes is used to gain conclusive information on the substances injected by users. It enables monitoring local trends as well as multi drug use and adulteration, possibly leading to elevated risks of adverse health consequences and overdose deaths (European Syringe Collection and Analysis Project Enterprise, European Union Drugs Agency). In forensic toxicology, drugs of abuse screening - whether in body fluids, drug paraphernalia or substance samples - is usually carried out by gas chromatography or liquid chromatography coupled to mass spectrometry. Run times of these methods vary between 10 and 25 min, depending on the used approach.

Direct Analysis in Real Time (DART) has been demonstrated a suitable tool for the analysis of a variety of samples, especially in forensic chemistry. In recent years, it's applications in clinical or forensic toxicology have also increased. In combination with high resolution mass spectrometry (HRMS) this chromatography-free approach provides spectral information for targeted and untargeted screening within a very short analysis time of 20 to 30 seconds.

Objectives: The aim of the project was to evaluate a rapid screening workflow for drugs of abuse and adulterants in used syringes using DART-HRMS.

Methods: Syringes were collected at harm-reduction services and drug consumptions rooms in the city of Cologne, Germany and rinsed five times with 1 mL acetonitrile. For the conventional LC-MSn screening approach (Toxtyper 3.0, Bruker) the solution was diluted with LC eluent and analysis was performed with 11 min runtime. For identification an in-house generated spectral library containing 1,500 drugs and drugs of abuse was used. An additional analysis was performed using a Bruker JumpShot source coupled with a Bruker QTOF-MS operating in auto MS/MS mode, delivering results in just 20 s analysis time per sample. Therefore, the rinsing solution was diluted 1:10 with acetonitrile. Compound identification and reporting were streamlined through an automated spectral library search (Seized Drug Suite, Bruker) using in-house and 3rd party HR-MS libraries containing 400 to 5,000 compounds.

Results: As expected, heroin, cocaine or a combination of the two were the substances most frequently injected by this clientele. In addition, there were a small number of amphetamine, MDMA, methadone, and methylphenidate findings. Heroin was typically adulterated with caffeine and paracetamol. The poppy alkaloids papaverine and noscapine as well as acetylcodeine and 6-acetylmorphine were also detectable in various combinations and can be used to verify heroin findings. As already seen in recent studies, the injected cocaine seems to be of high purity, with only about 8 % of the samples adulterated with phenacetin.

Adjustment of the dilution step led to DART-HRMS results that were in good agreement with data from the routine screening and no false positive results were received. Screening time for 200 to 250 samples usually sent in per collection could be reduced from several days to a few hours.

Discussion: Analysis of syringe residues offers an additional and complimentary view on the compounds injected within a certain community of drug users. The combination of DART with HRMS allows for a fast analysis of drug paraphernalia and a quick identification of compounds using targeted or un-targeted workflows. The workflow allows the use of third-party spectral libraries and the acquired HR-MS data can be used to identify unknowns. Depending on the analytical question, e.g. differentiation of isobaric compounds, a confirmatory analysis (e.g. LC-QTOF-MS) is recommended. Nevertheless, due to the unmatched speed, DART-HRMS is especially suited for rapid screening analysis, either as a stand-alone method or as guidance tool for subsequent in-depth analysis.

Conflict of Interest

Some of the authors are employees of Bruker Daltonics Inc.

P-083

Advanced Analysis of Controlled Substances Using DART-TIMS-QTOF Mass Spectrometry

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Abstract

Introduction: The use of Direct Analysis in Real Time (DART) with Quadrupole Time of Flight-Mass Spectrometry (QTOF-MS) is praised for its ability to generate highly informative data quickly and easily. This makes it particularly suitable for meeting the high throughput demands often required in the analysis of controlled substances. For isomeric compounds however, DART-QTOF-MS is limited when fragmentation patterns are not significantly different or several isomers are present in one sample leading to mixed fragmentation spectra. Combining DART-QTOF mass spectrometry with Trapped Ion Mobility Spectrometry (TIMS) is a promising approach to overcome this limitation. TIMS allows separation of ions of different shapes and sizes in the gas phase and is therefore a powerful post-ionization tool for isomer separation.

Objectives: This study investigates the chromatography-free separation of isomeric drugs of abuse using TIMS coupled with DART-QTOF mass spectrometry.

Methods: A DART JumpShot source (Bruker Daltonics) and a timsTOF mass spectrometer (Bruker Daltonics) were used for this study. 3 µL aliquots of drug mixtures including opioids, benzodiazepines, amphetamines as well as isomeric substance pairs were deposited onto a QuickStrip™ sample card. Full scan and parallel accumulation serial fragmentation (PASEF) MS/MS spectra were acquired in positive ionization mode. During PASEF mode, ion mobility separation and precursor selection for MS/MS are synchronized, resulting in fast MS/MS spectra acquisition with high precursor coverage. MS/MS spectra were searched against in-house libraries containing about 280 drugs and toxins as well as 3rd party libraries, e.g., NIST. Acceptance criteria were exact masses (< 5 mDa deviation) of precursor and fragment ions and the match of fragmentation patterns. In addition, helium and nitrogen were evaluated as potential ionization gases to determine their suitability for seized drug analysis.

Results: Up to 20 different drugs could be separated and identified in one sample. The successful separation of isomeric substance pairs including morphine and norcodeine as well as hydromorphone and norhydrocodone was confirmed by the presence of two nearly baseline separated mobility peaks in the mobilograms. Using PASEF mode, clean MS/MS spectra were yielded which then were used for library search-based identification. Depending on their size and shape, ions have a characteristic collisional cross section (CCS) in the gas phase. CCS values were calculated based on the measured inversed ion mobility and used as additional identification criterion to assist in the identification of isomeric and non-isomeric compounds. A CCS deviation from the measured to the theoretical value of 2% (narrow) to 5% (wide) was accepted. The results also demonstrate that mobility filtering increases the signal-to-noise ratio and improves the spectral quality. The comparison of the gases showed that the use of helium led to a higher sensitivity.

Discussion: Adding TIMS to DART-QTOF-MS is considered a promising approach as it allows for the separation of isomeric drugs while maintaining the high speed and chromatography-free approach powered by DART. The main benefits heighlighted by this study are the separation of isomers as well as improved spectral quality and generation of CCS values for increased confidence for compound identification.

Conflict of Interest

Some of the authors are employees of Bruker Daltonics Inc.

P-084

Drug Screen Suite: A Simplified but Comprehensive Solution for Toxicological Routine Screening Using Liquid Chromatography - High Resolution Mass Spectrometry

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Abstract

Introduction: Due to the heavy workload in forensics, robust and easy-to-use solutions with a high degree of automation in data handling are essential, especially since screening evaluation can be a significant bottleneck considerably delaying case work. In addition, the emergence of new psychoactive substances (NPS) calls for an option of ongoing and timely update of screening methods.

Liquid chromatography - high resolution mass spectrometry (LC-HRMS) is one of the most comprehensive screening techniques in forensic toxicology. Retention time, the exact mass of the compound and its high resolution MS/MS spectrum allows for a reliable identification of drugs and their metabolites. To tackle the need for constant updates, two strategies are pursued: Firstly, if reference material is at hand, spectra of new substances can easily be recorded and added to the library using an included workflow. Secondly, the integrated open library principle allows the integration of commercial or open-source third-party libraries.

Objectives: We present the evaluation of a comprehensive and highly automated UHPLC-HR-MS/MS spectral library screening method. This method is designed to detect and confirm both parent drugs and metabolites in various matrices like urine, serum, postmortem blood, and vitreous humor.

Methods: About 120 drugs and drugs of abuse, e.g. amphetamines, benzodiazepines, NPS, antidepressants, and opiates were spiked in three different blank urine samples (V = 100 µL) at concentration levels down to 1 ng/mL and subsequently analyzed after precipitation with cold acetonitrile. In addition, extracts of serum, postmortem blood, and vitreous humor from case work were reanalyzed. Chromatographic separation was performed with a twenty minutes gradient (A: 0.1 % formic acid, B: methanol) on an Intensity Solo 1.8 C18-2 100 x 2.1 mm column and AutoMS/MS spectra were acquired in positive mode on a Bruker QTOF-MS. Acquired spectral data was matched against a library of currently around 400 substances of toxicological relevance and the Maurer/Meyer/Helfer/Weber (MMHW, Wiley-VCH) LC-HR-MS/MS library of drugs poisons and their metabolites containing more than 5,000 compound entries. Data processing and reporting was carried out by an automated algorithm.

Results: Using the Drug Screen Suite with MS^2 spectral library searching – including retention time matching and exact masses (< 5 mDa deviation) of precursor and fragment ions – 80 % of the spiked compounds in urine were reliably identified at levels of 10 ng/mL or lower, with only few false positives.

With the exception of norbuprenorphine, all substances suitable for positive ESI were successfully detected in proficiency tests for the analysis for driver fitness determination. Urine samples from drug screening proficiency tests as well as serum samples from therapeutic drug

monitoring proficiency tests could also be analyzed successfully with the exception of GHB and norbuprenorphine.

Various samples from forensic case work, including serum, postmortem blood, blood clot, urine, and vitreous humor were used to test this workflow with real-life samples and screening results were in accordance with the overall findings of the respective case. Beside the parent compound, AutoMS/MS in combination with the MMWH library led to the additional detection of metabolites, which can be used as additional confirmation of the intake of a substance. Use of HR data helped to reduce the number of false positives, especially in samples with a high matrix load.

Discussion: Using the Drug Screen Suite solution enables the rapid detection and identification of compounds in a single run. The open library concept in combination with automated data processing and reporting helps to reduce the turnaround time in case work and facilitates the implementation of HRMS into the routine workflow of a toxicological laboratory.

Conflict of Interest

Some of the authors are employees of Bruker Daltonics Inc.

P-085

One Month Snapshot of the Prevalence of Anti-Epileptic Drugs in Central Virginia

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Abstract

Introduction: In 2007, recommendations for testing drug-impaired driving and motor vehicle fatality cases with standardized analytes and their cutoffs were published based upon responses to a survey sent to select laboratories. Updates to these recommendations were published in 2013, 2017, 2021, and 2025 based upon responses to subsequent surveys sent to select laboratories. From the first update in 2013, gabapentin became a recommended analyte for Tier II testing. Laboratories taking the survey suggested moving gabapentin to the Tier I list beginning in the 2013 update and this was done in the 2025 update. In a recent webinar hosted by the Society of Forensic Toxicologists (SOFT), the recommended cutoff concentration for gabapentin was established as 1000 ng/mL. With this information, the Virginia Department of Forensic Science (DFS) conducted a preliminary prevalence assessment of common anti-epileptic drugs (AEDs) including gabapentin. Many of these AEDs are included in the Tier II testing recommendations.

Objectives: The purpose of this study was to perform a preliminary evaluation of the prevalence of AEDs present in casework in Central Virginia.

Methods: The typical workflow for testing toxicology cases at DFS, regardless of case type, includes an alcohols test (ethanol, methanol, isopropanol, and acetone) and drug screens (requested or assigned, depending on case type). The case types for which biological specimens are received for toxicology are Implied Consent Driving (DUI/DUID), Toxicology-Other (TO), and Postmortem (ME). TO cases are frequently hospital-collected samples and include, but are not limited to, a variety of case types, such as driving under the influence, child endangerment, and drug facilitated crimes.

When cases are analyzed for alcohols, a small volume is typically poured into a secondary vessel before aliquoting (“pour offs”), though cases with limited sample volumes are not poured off to conserve sample. Over the course of four weeks (Mar 10 – Apr 4, 2025), the de-identified pour-off remainders from all indicated case types were subsequently screened for the following AEDs: gabapentin, pregabalin, levetiracetam, lamotrigine, zonisamide, licarbazepine, oxcarbazepine, carbamazepine, topiramate, phenytoin, and lacosamide. For screening, 100-200 µL of sample is fortified with internal standard (IS), followed by protein precipitation with 1 mL of methanol. The centrifuged extract is transferred to autosampler vials for injection on a Liquid Chromatograph Tandem Mass Spectrometer. The administratively set limit of detection (LOD) for all analytes is 500 ng/mL, and the administratively set quantitative range is 1,000 – 40,000 ng/mL.

Results: Over the 4-week test period, 106 DUID, 23 TO, and 126 ME samples were tested for alcohols. The 255 cases screened for alcohols during the study period represent ~9% of the projected caseload for DFS’ Central Toxicology section in 2025. Of the 255-case total, 103 DUID, 3 TO, and 87 ME samples were screened for AEDs.

	DUID	TO	ME
Samples Screened for AEDs	97.17%	13.04%	69.05%
Samples Indicating AEDs (> LOD)	1.89%	0%	7.14%
Samples Indicating Gabapentin	0.94%	0%	3.97%

Conclusion/Discussion: With the move of gabapentin to Tier I, a preliminary 4-week prevalence study was performed in the Central Virginia region. AEDs (including gabapentin) were indicated in 1.89% of DUID cases and 7.14% of ME cases. Of the ME cases in which AEDs were detected, ~78% were samples from decedents found unresponsive or otherwise suspected to have overdosed. Approximately 71% of this subset of samples also contained opioid class drugs in combination with stimulants and/or central nervous system (CNS) depressants, and the remaining 29% of these cases contained either opioid class drugs or multiple CNS depressants. Based upon the low prevalence observed within the preliminary one-month test period, further periodic monitoring may be beneficial prior to a full workflow adjustment.

P-086

Unrealized Gains: Toxicology HPLC-MS/MS Method Optimization Using a Multi-factorial Approach in Oral Fluid and Urine Matrices

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Abstract

Introduction: Although toxicology laboratories routinely refine foundational HPLC–MS/MS parameters, many advanced instrument settings that could substantially enhance analytical performance and sensitivity remain underexplored. Current optimization efforts focus on chromatographic conditions and basic mass spectrometer variables, while sophisticated configurations in modern systems are largely overlooked. As a result, laboratories often rely on legacy operating conditions without systematically evaluating the broader range of instrumental options. This study addresses this gap by examining a wider array of instrumental variables than typically considered during routine method development on contemporary mass spectrometry platforms for oral fluid and urine matrices. Ultimately, targeted adjustment of advanced settings is shown to significantly improve sensitivity and overall method robustness.

Objectives: The primary objective was to evaluate analytical gains achievable through systematic HPLC-MS/MS parameter optimization compared to manufacturer default conditions on Sciex and Shimadzu systems. Specific aims included: (1) assessing effects of mobile phase additives on ionization efficiency and chromatographic performance; (2) optimizing electrospray ionization source parameters including gas flows, temperatures, and voltages; and (3) conducting compound-specific optimization of multiple reaction monitoring (MRM) transitions and collision energies using non-standard protocols versus automated optimization. Secondary objectives were to quantify sensitivity improvements, evaluate method robustness, and develop optimization protocols applicable to various forensic analyte classes and sample matrices.

Methods: A systematic multi-factorial approach using JMP® (SAS Institute Inc., Cary, NC) was applied to oral-fluid and urine matrices, assessing > 40 analytes across representative drug classes including stimulants/illicit drugs, opioids/opiates, benzodiazepines, non-benzodiazepine hypnotics, antidepressants, barbiturates, antipsychotics, anticonvulsants, anesthetics/dissociatives, muscle relaxants, and ethanol biomarkers. Mobile-phase optimization evaluated common volatile organic acids, their ammonium salts, and ammonium fluoride using a definitive screening design (DSD). Source-parameter optimization on Shimadzu platforms employed response-surface methodology (RSM) to refine nebulizing-gas flow, heating-gas flow, interface temperature, DL temperature, heat-block temperature, and drying-gas flow, while equivalent parameters were examined on the Sciex 4500 for ethanol biomarkers. Interface-voltage optimization used a custom experimental design for the 8060NX and a single-factor design for the 8050, which lacks the ion-focus voltage available in the NX source. Compound-specific parameters—including collision energy and MRM transitions—were optimized via either manufacturer-automated fragment selection or stepwise manual adjustment on both platforms. Performance was evaluated by integrated area counts, signal-to-noise ratios, limits of detection and quantification (LOD/LOQ), linearity, precision, accuracy, and assessment of ion suppression or enhancement.

Results: Optimized conditions showed significant improvements over default parameters. Analyte area counts increased by approximately 1.3 to more than 10-fold across target analytes, with source settings and interface/ion focus voltages providing the most significant gains. Mobile phase optimization confirmed acetic acid as the preferred phase modifier when negative mode analysis was included, while ammonium fluoride improved positive mode analysis but suppressed negative mode signals. Source parameter optimization identified nebulizing gas and heating gas flow as most critical, with optimal drying gas flow differing significantly between the 8060NX and 8050 platforms. Interface voltage showed significant improvements compared to default tune file settings on both Shimadzu platforms, and optimal ion focus parameter setting diverged significantly from the default setting for the 8060NX. Compound-specific MRM transition and filter settings resulted in average signal enhancement of 5-30% compared to default values.

Discussion: The multi-factorial optimization approach yielded substantial analytical improvements over manufacturer defaults, revealing unrealized potential in routine toxicology methods. Sensitivity improvements exceeding 10-fold for some analytes have direct implications for detecting lower analyte concentrations in forensic samples, potentially expanding detection windows for investigative purposes. This systematic approach provides a framework for forensic laboratories to enhance existing methods without requiring new instrumentation, underscoring the importance of moving beyond default conditions toward evidence-based method optimization in forensic toxicology.

P-087

Evaluation of the Performance of the ARK™ Fentanyl II Assay

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Abstract

Introduction: Fentanyl is a potent opioid mu receptor agonist that is available for therapeutic use in several formulations for the treatment of acute and chronic pain. Fentanyl has been detected in the illicit drug supply in the United States for many years but has gained attention recently due to its frequent appearance in illicit drugs, such as heroin, cocaine, methamphetamine, and the synthesis of fentanyl analogs. In the clinical laboratory, rapid identification of fentanyl and its metabolite norfentanyl in biological specimens is important for the successful treatment of individual users. The ARK™ Fentanyl II Assay is a homogeneous enzyme immunoassay (IA) that may serve as a screening tool for presumptive drug testing that can be confirmed by definitive liquid chromatography-tandem mass spectrometry (LC-MS/MS)-based testing.

Objectives: To evaluate a rapid screening tool for fentanyl. We modified the ARK™ Fentanyl II Assay for human urine and compared the results with an LC-MS/MS assay.

Methods: The ARK™ Fentanyl II Assay (ARK Diagnostics, Inc., Fremont, CA) was modified by diluting the vendor-provided cutoff calibrator from 1.0 ng/mL to 0.5 ng/mL and low- and high-quality control materials from 0.5 ng/mL to 0.25 ng/mL and 1.5 ng/mL to 0.75 ng/mL, respectively. The assay was validated on a Beckman Coulter AU 5800 series chemistry analyzer by determining accuracy, carryover, and precision around the cutoff. Interference was assessed by testing approximately 150 compounds at a concentration of 1000 ng/mL.

All presumptive positive screening results were assayed by a definitive CLIA-validated LC-MS/MS assay with a Prelude LC (Thermo Fisher Scientific, Waltham, MA) and a SCIEX 6500 triple quadrupole MS (SCIEX, Marlborough, MA). A Kinetex Biphenyl 50 x 3.0 mm 2.6 um 100A column with a gradient binary mobile phase of 0.1 % formic acid in deionized water and 0.1% formic acid in methanol was utilized. The lower reporting limit for fentanyl and norfentanyl was 0.5 ng/mL. A total of 251 random de-identified urine specimens were tested with both the IA and LC-MS/MS methods.

Results: The precision of the IA around the cutoff of 0.5 ng/mL for fentanyl demonstrated a within-run coefficient of variation of <15%. No carryover was observed at a fentanyl concentration of 1,000 ng/mL. Over-the-counter medications, amphetamines, antidepressants, antihistamines, anticonvulsants, barbiturates, benzodiazepines, opioids, and prescription fentanyl derivatives (alfentanil, remifentanil, and sufentanil) did not produce positive IA responses. The fentanyl analogs acryl fentanyl, acetyl fentanyl, butyryl fentanyl, furanyl fentanyl, 3-methyl fentanyl, and cyclopropyl fentanyl produced positive responses.

In comparison with LC-MS/MS confirmation, the ARK™ Fentanyl II Assay achieved an overall accuracy of 93.0%. Sensitivity was 82.7%, specificity was 98.8%, positive predictive value was 97.6%, and negative predictive value was 90.0%. All IA false negatives were due to samples containing only norfentanyl at concentrations <5 ng/mL.

Discussion: The ARK™ Fentanyl II Assay provides high specificity and strong performance as a screening tool for fentanyl in urine, with minimal interference from other drugs. Fentanyl analogs may produce a positive IA response. Users of this IA should be aware of the limitation of this assay which is the lack of ability to detect low concentrations of norfentanyl.

P-088

Detection Rates of Fentanyl in Oral Fluid and Urine Specimens in the North Region of the United States

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Abstract

Introduction: Illicit fentanyl use remains a serious public health concern. Drug monitoring is a key component of treatment protocols in substance misuse programs. Extensive surveys and publications on the presence of fentanyl in patient urine specimens provide valuable insights into drug use across different geographical regions and among various patient populations. Urine is the preferred specimen for monitoring fentanyl, while oral fluid (OF) serves as an alternative testing matrix to detect more recent exposure.

Objectives: To determine the rates of fentanyl positivity in both urine and OF specimens tested using LC-MS/MS in the northern region of the United States and to evaluate these rates based on the clinical specialty of the specimens.

Methods: This retrospective study included data from random OF and urine specimens analyzed during the third quarter at Quest Diagnostics for the New England region (tested in Marlborough, MA [MARL]) and the greater Pittsburgh area (tested in Pittsburgh, PA [PITTS]) using CLIA-validated LC-MS/MS assays. Specimens were categorized based on the ordering clinical specialty, which included drug rehabilitation facilities, primary care providers, pain management, and psychiatry. Additionally, individuals who were underinsured or uninsured and qualified for federal assistance were included. The miscellaneous group consisted of specimens that could not be placed into other categories. The data was analyzed for values at or above the lower reporting limits for positive results: 0.1 ng/mL for fentanyl in OF and 0.5 ng/mL for both fentanyl and norfentanyl in urine. Statistical analysis was conducted using the chi-square test.

Results: The fentanyl test results included 8,230 OF specimens and 107,510 urine specimens. The overall positive rate for fentanyl was significantly higher in MARL compared to PITTS, with 37.8% in OF and 14.4% in urine for MARL, versus 9.3% in OF and 7.0% in urine for PITTS (Table).

In MARL, the highest fentanyl positivity rates in OF were seen in psychiatry (55.2%) and drug rehabilitation (53.9%), while the highest rates in urine were seen in drug rehabilitation (20.8%) and federal assistance health centers and primary care providers (each 9.7%). In PITTS, the highest positivity rates in OF were seen in federal assistance health centers (22.3%) and drug rehabilitation (11.0%), while the highest rates in urine were seen in drug rehabilitation (9.7%) and federal assistance health centers (7.9%).

Note: The positive rate (%) refers to the percentage of positive fentanyl related to the total tests in each group. The miscellaneous group is excluded from discussion due to the variability of specimen sources.

Discussion: The overall rates of fentanyl positivity in OF and urine were significantly higher in MARL compared to PITTS. This suggests that fentanyl misuse is more prevalent in New England than in the greater Pittsburgh area, potentially due to greater availability of illicit fentanyl in the New England region. The high positivity rates among specimens from drug rehabilitation centers in both OF and urine highlight the need for increased resource allocation for individuals seeking treatment for substance use disorders. Furthermore, the data suggests that those seeking healthcare who are uninsured or underinsured should be a targeted population for treatment and prevention efforts related to fentanyl misuse. The reasons for the high fentanyl positivity rate in OF specimens from psychiatry specialists for MARL warrant further investigation.

P-089

Repurposing Validated Forensic Toxicology Methods for Quantitating Gamma-Hydroxybutyrate (GHB) in Fermented Alcoholic Beverages

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Abstract

Introduction: Gamma-hydroxybutyrate (GHB) is used to adulterate beverages in drug-facilitated sexual assaults (DFSA), and the presence of low endogenous levels found in many drinks require quantitative analysis to determine if products have been purposefully adulterated.[1]Unfortunately, many forensic toxicology laboratories are not validated for GHB quantitation in beverages. This study adapts an analytical platform previously validated for GHB testing in human urine[2] to quantitate GHB in alcoholic beverages and provide a foundation for the simultaneous testing of human urine and beverages.

Objectives:

- Adapt a validated LC-MS/MS forensic toxicology method to quantitate GHB in beverages.
- Test robustness by evaluating endogenous GHB levels in fermented beverages obtained from local sources.
- Demonstrate forensic value through the examination of evidence collected during DFSA investigations.

Methods: Samples were processed using commercially available ToxBox® 96-well plate forensic test kits preloaded with GHB calibration standards (0.5–100 mg/L), second source quality control (QC) standards (1.5, 6.0, and 30 mg/L), and a deuterated internal standard (GHB-d_6, 5 mg/L). Test kits also provide a premanufactured ammonium hydroxide sample modifier (Solution A), an organic extraction solvent (Solution B), and diluent for reconstitution (Solution C). Standards and QCs were prepared with the addition of 100 µL of water to wells containing standards and internal standard. 100 µL of unknown samples were added to wells that only contained internal standard. Standards, QCs, and samples were modified with the addition of 100 µL of Solution A and further diluted with 300 µL of water. All samples were mixed (500 rpm) for 5 min at room temperature prior to the addition of 650 µL of Solution B. These mixtures were aspirated and dispensed 10X and allowed to separate for 10 min. The lower aqueous layer was removed prior to drying the remaining organic phase under nitrogen at 40°C. Wells were reconstituted with 200 µL of Solution C. Quantitative analysis was performed on a Bruker EVOQ-DART-TQ+ LC-MS/MS equipped with an ESI source using optimized conditions for GHB confirmation testing.

Robustness was tested by comparing endogenous GHB levels with previous reports and evaluating matrix interferences across nine different brands representative of red wine, white wine, and champagne. Forensic value is illustrated through the evaluation of champagne evidence collected as part of DFSA investigations.

Results: LC-MS/MS analysis provided a linear response (r^2 = 0.998) across the analytical measurement range (0.5–100 mg/L). Evaluation of quality control samples showed that

the analytical procedure is accurate (95% to 106% bias) and precise (1% to 8% RSD). No interferences were observed in any matrix. Endogenous GHB levels found in red wine (10 to 25 mg/L), white wine (5 to 7 mg/L), and champagne (2 to 3 mg/L) were comparable to previously reported concentrations.[1] The concentration of GHB found in the champagne sample collected as part of DSFA investigations was similar to endogenous levels found in three control champagne samples (approximately 3 mg/L). GHB testing in blood and urine was not performed in this case. Data suggest the evidence was not purposefully adulterated with GHB.

Discussion: This study shows the importance of quantitating GHB in beverages collected as part of DSFA investigations. The new LC-MS/MS analytical procedure is suitable for quantitative GHB testing in fermented beverages. The analytical measurement range is sufficient to distinguish endogenous levels from concentrations expected to be found in adulterated beverages.[1] The streamlined analytical approach and use of forensic test kits can assist forensic laboratories with limited resources and support the simultaneous testing of toxicology and product adulteration samples.

Citations

1. Elliott and Burgess, *Forensic science international* (2005): 289-92.
2. Avram *et. al., Chemical research in toxicology* (2023): 1584-1591.

Conflict of Interest

Some authors are employees of PinPoint Testing, LLC

P-090

When Xanax Isn't Xanax: A Fatal Benzodiazepine Toxicity Involving Bromazolam and Desalkylgidazepam from Suspected Counterfeit Alprazolam

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Abstract

Introduction: Over the past decade and a half, novel psychoactive substances (NPS) have increased in prevalence, often appearing in misrepresented and/or adulterated products. NPS benzodiazepines are the most frequently detected NPS in toxicological casework and pose significant public safety threats, as they are commonly masked as traditional benzodiazepines like alprazolam. These substances are often found in combination with other drugs, which enhances their risk for toxicity. Bromazolam and desalkylgidazepam are among the latest NPS benzodiazepines to emerge on the market following the scheduling of other compounds in their class. Here we report a case involving a 22-year-old male found unresponsive at home, foaming at the mouth, after being found responsive in bed hours earlier snoring. The decedent began using 'Xanax' recreationally to help "relax" in the months prior to his death and had a documented history of frequent 'Xanax' use and hospitalization in the days immediately preceding his fatality.

Objectives: To present the findings of a fatal benzodiazepine toxicity involving bromazolam, desalkylgidazepam, and suspected illicit Xanax use. Although uncommon, this case report highlights the potential toxic profile of NPS benzodiazepines and benzodiazepines often considered safe when used without other drugs.

Methods: Postmortem specimens were collected at autopsy at the Travis County Medical Examiner (TCME) in Austin, TX. Femoral postmortem blood was screened for volatiles using headspace dual-column gas-chromatography with flame ionization detection, and for drugs of abuse via immunoassay (ELISA) targeting four drug classes. Comprehensive qualitative drug screening was performed using a validated liquid-chromatography time-of-flight mass spectrometry (LC-QTOF-MS) method following an acetonitrile protein precipitation. The assay screened for over 350 drugs with a 10 ng/mL limit of detection for all benzodiazepines, including bromazolam and desalkylgidazepam. Presumptive positive findings were confirmed and quantitated using validated liquid chromatography tandem mass spectrometry (LC-MSMS) methods following alkaline liquid-liquid extraction. Quantitative analysis of desaklylgidazepam in femoral blood was performed by a reference laboratory.

Results: Autopsy findings revealed a well-developed, morbidly obese (BMI >50 kg/m2) male with an enlarged heart (580g), left ventricular hypertrophy, & edema of the lungs. Routine toxicology was presumptive positive for cannabinoid metabolites in the femoral blood. Quantitative LC-MSMS analysis identified the following concentrations in postmortem femoral blood: bromazolam (220 ng/mL), desalkylgidazepam (66 ng/mL), alprazolam (<20 ng/mL), and 7-aminoclonazepam (<20 ng/mL). Based on the autopsy, investigative, and toxicological findings, the cause of death was certified as toxic effects of bromazolam, desalkylgidazepam, alprazolam & clonazepam, with the manner ruled accidental.

Discussion/Conclusion: This case highlights the emerging threat of novel benzodiazepines

in fatal drug toxicity, particularly when misrepresented as traditional prescription medications like alprazolam. Notably, this represents the highest bromazolam blood concentration and the first desalkylgidazepam identification observed at the TCME to date. Although benzodiazepines are widely regarded as having low toxicity profiles when used in isolation, this case highlights an uncommon but important exception. While limited pharmacological data exist, anecdotal reports suggest that bromazolam has a longer duration of action and may be similarly or more potent than other benzodiazepines such as alprazolam and diazepam. This case suggests that benzodiazepine toxicity alone may be fatal, especially in vulnerable individuals with significant comorbidities like obesity and in cases involving NPS benzodiazepines. While current analyses did not identify other contributing substances, the presence of an undetected compound cannot be entirely excluded. As NPS prevalence continues to rise, forensic toxicologists must remain vigilant in detecting and interpretating NPS benzodiazepines, even in the absence of polydrug use. Expanding toxicology testing scopes and improved NPS surveillance are critical to better understanding the evolving role of NPS in public health and mortality.

Keywords

bromazolam, postmortem toxicology, NPS benzodiazepines

P-091

Prevalence of Medetomidine, Xylazine, and their Metabolites in an Addiction Medicine Setting.

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Abstract

Introduction: The emergence of medetomidine as a new adulterant in the U.S. illicit drug supply poses a potential public health crisis. Like the veterinary medicine xylazine, medetomidine (and its d-isomer dexmedetomidine) are both potent α2-adrenoreceptor agonists. Medetomidine has recently been detected in opioid overdose cases, is 200–300 times more potent than xylazine, and lacks evidence-based treatment protocols for its toxicity. It remains unclear whether medetomidine is replacing xylazine in the drug supply or if both will coexist in the illicit drug supply.

Objectives: Here we investigate the prevalence of medetomidine, xylazine and their respective metabolites 3-hydroxy medetomidine and 4-hydroxy xylazine in urine samples from an addiction medicine setting along with other illicit drugs routinely monitored.

Methods: A total of 27,152 urine samples from addiction medicine clinics were analyzed between 2024-12-19 and 2025-05-05. Testing was performed using the ToxAssure® Comprehensive Profile (Labcorp MedWatch®), quantifying 48 targets via ultra-high-performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS) on Sciex 5500 platforms. Study samples additionally tested for xylazine, medetomidine, 4-hydroxy xylazine, and 3-hydroxy medetomidine with a qualitative cutoff of 2 ng/mL. The identity of all detected targets were verified via transition ratios. All analyses were IRB-approved. After testing, data were processed using ASCENT (Indigo BioAutomation), stored in an AWS database, and analyzed in R.

Results: We observed that medetomidine was more prevalent than xylazine in the patient population, while the inverse was true for 3-hydroxy medetomidine versus 4-hydroxy xylazine. Out of the sample set, >9% (2459) were positive for medetomidine, compared to 5.5% (1485) positive for xylazine. In contrast, only 0.9% (235) of samples were positive for 3-hydroxy medetomidine, while 1.9% (430) tested positive for 4-hydroxy xylazine.

Medetomidine and xylazine are believed to be primarily utilized as adulterants added to fentanyl, but from a body burden perspective, users' may be concurrently exposed to other illicit drugs and opioids. To assess the prevalence of synthetic opioids in the presence of medetomidine and/or xylazine, we analyzed for co-positivity rates with fentanyl, norfentanyl, and 6-monoacetylmorphine (6-AM). Our results indicate that while xylazine, 4-hydroxy xylazine, and 3-hydroxy medetomidine display similar co-positivity rates, medetomidine displays lower rates. For example, xylazine, 4-hydroxy xylazine, and 3-hydroxy medetomidine display fentanyl and norfentanyl co-positivity rates of 95-100%, while medetomidine displayed a 27% positive rate with fentanyl and norfentanyl. The frequency of detecting urine medetomidine in the absence of fentanyl or other opioids warrants investigation.

The overlap between samples positive for both medetomidine and xylazine was relatively small at 538 samples (2.0%). The samples positive for both xylazine and medetomidine displayed co-positivity rates with fentanyl like the co-positive rate of xylazine with fentanyl.

It is also unclear if users of heroin may also be exposed to medetomidine or xylazine. Samples containing the diagnostic urinary metabolite 6-AM were queried to determine the overlap with detecting medetomidine or xylazine. Our results indicate that out of the small percentage of samples positive for 6-acetylmoprhine (1.3%), about a third (33.1%) were also positive for medetomidine and about two-thirds (71.4%) positive for xylazine. This compares to 88.9% of overlap between 6-AM and fentanyl.

Discussion: Our results suggest in late 2024 through early 2025, medetomidine is in higher prevalence than xylazine in our patient population. Out of 27,152 unique patient specimens, we showed that almost double the number of samples were positive for medetomidine (9.1%) compared to xylazine (5.5%). These results could indicate that the illicit drug market may be transitioning from xylazine to medetomidine and has implications for public policy and health providers.

P-092

Determining the Detectability and Potential Target Epitope of 212 Fentanyl Analogs and Synthetic Opioids using the Abbott iSCREEN™ Urine Test DX Drug Screen Tox Cup

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Abstract

Introduction: The United States continues to be plagued by the opioid epidemic including the fourth wave in which fentanyls are still highly prevalent. Detectability of fentanyl analogs and synthetic opioids continues to be of interest to clinical and forensic laboratories. The US Centers for Disease Control and Prevention (CDC) coordinated production of the Traceable Opioid Material® Kits (TOM Kits®), which has assisted laboratories in assessing the ability of commercially available screening methods to detect current and potentially emerging opioids.

Objectives: Evaluate the ability of CLIA Waived iSCREEN™ Urine Test DX Drug Screen Tox Cup to detect current and potentially emerging fentanyl and opioid analogs, and known potential interferents. Determine the likely target epitope in relation to previously presented and published studies using commercially available homogeneous immunoassays. The final data analysis of these >300 evaluations will also include this prediction.

Methods: From the TOM Kits®, 212 opioids were evaluated, which included 195 fentanyl analogs and 17 synthetic opioids. Additionally, 6 potential interferents (bilirubin, biotin, ciprofloxacin, m-CPP, trazodone, and uric acid) were evaluated at elevated physiological concentrations. The compounds were analyzed using a recently available CLIA-Waived commercial point-of-care urine drug screening kit (iSCREEN™ Urine Test DX Drug Screen Tox Cup, Abbott Diagnostics) (Cup). The detectability of the opioids was initially evaluated by preparing each compound individually in drug-free urine at 10 ng/mL and then analyzing the prepared solution to determine if the analog screened positive at the Cup's fentanyl cutoff (1 ng/mL) in singlicate. If the screen result was positive, no further testing was performed for that opioid. If the result was negative, the analog was prepared at a 100 ng/mL concentration and analyzed using a new Cup. Opioids not testing positive at 100 ng/mL were considered not detectable. The difference in reactivity of analogs with the Cup was evaluated in conjunction with the chemical structure of each opioid. Due to the high concentrations of norfentanyl (major fentanyl metabolite) commonly detected in urine, a 1000 ng/mL norfentanyl solution was also evaluated. The potential interfering substances were tested at >=10,000 ng/mL.

Results: The Cup was able to detect 125 of the 212 opioids, 92 opioids at 10 ng/mL and 33 opioids at 100 ng/mL. Eighty-six opioids were not detectable by the Cup. Norfentanyl was not detectable at the three prepared concentrations. The six interferents prepared at an elevated physiological concentration were not detected. Three synthetic opioids were only detectable by the Cup (3,4-Ethylenedioxy U-47700, U-48753E, U-51754). When compared to previous data obtained using this same set of opioids evaluated for detectability using four commercial fentanyl immunoassays; of the 87 compounds that didn't test positive using the Cup, all but 8 were detected using other immunoassays. Five of the 8 had para-methoxy substitutions on the aniline ring.

Discussion: The Abbott iSCREEN™ Urine Test DX Drug Screen Tox Cup detected 59% of the opioids, 43% of analogs at 10 ng/mL and 16% of analogs at 100 ng/mL. Forty-one percent of the analogs were not detected at either concentration.

These results are similar to those of the same opioids evaluated using four commercially available homogeneous immunoassays, where the immunoassays were able to detect between 132 (62%) and 158 (75%) of the opioids. Eighty-one (38%) of the opioids were detectable by all 5 assays, at varying concentrations, and 25 (12%) were not detectable by any of the 5 assays.

Para-methoxy substitutions were poorly tolerated by the Cup with 5/8 not being detected and the other 3 only detected at the higher concentration.

This information will be of use in both clinical and forensic settings in evaluation of the potential false-positive or false negatives in urine fentanyl screening.

P-093

The Potential Forensic Implications of Latent Alcohol Exposure by Blood Donors

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Abstract

Introduction: Blood donations are an integral part of healthcare not only just for blood product replenishment from acute traumatic incidences (i.e. motor vehicle crashes and gunshot wounds) but also in the treatment of chronic illnesses (i.e. anemias and organ transplantations). The detection of alcohol or detection of latent alcohol use/misuse can have legal ramifications in human performance cases as well as compliance/abstinence cases, which are relevant in both acute incidences and chronic illnesses.

Due to the relatively short detection window of alcohol, latent alcohol use is detected by the presence of phosphatidylethanol (PEth), which is a direct biomarker of alcohol consumption (16:0/18:1 and 16:0/18:2 homologs). PEth is only formed due to the consumption of ethanol and can be used to detect latent ethanol use as it has a longer detection window. The presence of PEth in donated blood units can unknowingly have potential legal ramifications if transfused into an individual in both acute incidences and chronic illnesses, where compliance or abstinence is expected.

Blood donations are typically separated into different components for their critical role in the healthcare system. These components are transfused based on the recipient's clinical needs. Packed red blood cells (pRBCs) are a blood product prepared by separating the plasma and from erythrocytes, yielding a concentrated pRBC product. Detecting alcohol or identifying patterns of alcohol use or misuse is relevant in both emergency and long-term care settings. Due to alcohol's relatively short detection window, phosphatidylethanol's 16:0/18:1 and 16:0/18:2 homologs (PEth) are commonly used as biomarkers for ethanol ingestion. PEth concentrations ≥20ng/mL indicate alcohol use, while levels exceeding ≥200ng/mL of 16:0/18:1 suggest alcohol misuse. The presence of ethanol or PEth in donated blood products may have legal and clinical implications.

Objectives: This study aims to evaluate the presence and concentration of PEth in pRBCs from donor blood products; to ascertain the frequency and range of donor pre-donation alcohol exposure and the potential this exposure has on false legal implications.

Methods: Deidentified pRBC segments from donor units were obtained from the hospital's transfusion medicine service. A total of 400 units were analyzed for PEth using high-performance liquid chromatography-tandem mass spectrometry (HPLC-MS/MS). Donor units were analyzed using a previously presented protocol. Briefly, 100mcL of solution (calibrator, control, or donor unit) was mixed with 10mcL of internal standard (PEth 16:0/18:1-d5 and PEth 16:0/18:2-d5, 250 ng/mL), 100mcL of lysing buffer (water:acetonitrile, 80:20), vortexed, then loaded onto a Biotage ISOLUTE® SLE+ plates, and equilibrated for 5 minutes. PEth was eluted with three 700mcL aliquots of elution solution (ethyl acetate:isopropanol, 95:5), evaporated to dryness using a SPE Dry96, and reconstituted with 100mcL isopropanol. Analysis was conducted on a Waters TQ-S micro LC-MS/MS system using a Luna Phenyl-Hexyl column with a chromatographic gradient with a mobile phase of ammonium acetate and methanol/acetone (95:5).

The method was validated following ANSI/ASB Standard 036, including evaluations for linearity, precision, accuracy, selectivity, stability, and detection limits.

Results: Of the 400 pRBCs, 185 (46%) were PEth positive. PEth positive sample concentrations ranged from 10-1,412ng/mL for the 16:0/18:1 homolog and 10-1,709ng/mL for the 16:0/18:2 homolog. Of those, 33 (8%) had a PEth concentration ≥350ng/mL. Using American Red Cross provided calculations for pRBC components, a PEth concentration of ≥350ng/mL in a single donated pRBC unit could potentially result in a PEth concentration ≥20ng/mL in the transfused recipient.

Discussion: Of the pRBCs tested, 8% contained a sufficient PEth concentration to potentially yield a positive PEth result from a single transfused unit. Transfusing blood containing elevated PEth concentrations could result in a false positive indication of alcohol consumption or misuse in the recipient, which may have falsely prejudicial legal and clinical implications.

P-094

One Extraction, Sixty-Four Drugs: LC/MS/MS Oral Fluid Method Validation per NSC Drug Testing and ANSI/ASB 036 Standards

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Abstract

Introduction: High-throughput forensic toxicology laboratories require comprehensive testing panels to enhance efficiency and expand analyte coverage. The ability to confirm a broad range of substances through a single extraction streamlines workflow and improves productivity. Oral fluid has emerged as a preferred matrix in forensic toxicology due to its ease of collection and observed sampling. While an immunoassay-based screening method has already been validated by the laboratory, this study focuses on the development and validation of a confirmatory panel targeting 70 drugs of abuse. All analytes are extracted from oral fluid using a single liquid-liquid extraction, followed by analysis via LC-MS/MS employing three distinct MRM methods. Method validation was conducted in accordance with ANSI/ASB Standard 036 guidelines for forensic toxicology.

Objectives: To validate a comprehensive, confirmatory method for drug testing in oral fluid utilizing a single extraction.

Methods: A 1 mL sample of oral fluid/buffer (1:4) from the Quantisal® device was combined with 1 mL of isopropanol/internal standard mixture and 4 mL of 50:50 hexane:ethyl acetate. After 30 minutes of rotation and 30 minutes of centrifugation at 3.2 rpm, the top layer was split into two aliquots. Both aliquots were evaporated under nitrogen. One was reconstituted in 50:50 acetonitrile:water (Recon 1) and the other in 0.1% formic acid in water (Recon 2). Both were analyzed using LC-MS/MS on a Waters I-Class HPLC with a Xevo TQD. Recon 1 was injected onto a Cortecs C18 UPLC column (1.5 µm, 2.1 × 50 mm) with a 6.65-minute gradient for THC and synthetic cannabinoids. Recon 2 was injected onto an Acquity UPLC BEH C18 column (1.6 µm, 2.1 × 50 mm) with a 7.40-minute gradient using two MRM methods for other drugs of abuse. Method validation adhered to ANSI/ASB Standard 036 for forensic toxicology.

Results: Seventy drugs were targeted for validation in oral fluid; however, only 64 were successfully validated due to low recovery and sensitivity achieved for certain analytes (barbiturates, norbuprenorphine, norfentanyl, and THC metabolites). These analytes were also not part of the Tier 1 NSC recommendations, and therefore, not validated. Matrix effect studies indicated no significant interferences affecting analyte identification, though some compounds exhibited unacceptable ion suppression or enhancement. Additional matrix samples were fortified at the limit of detection to ensure no deleterious effects on the sensitivity occurred as a result. No carryover was observed in matrix blanks following high-concentration injections. Validated limits of detection ranged from 0.5 ng/mL to 200 ng/mL, with all analytes meeting or exceeding the 2021 National Safety Council (NSC) guidelines, except for benzoylecgonine, which had a cutoff of 25 ng/mL versus the recommended 8 ng/mL.

Discussion: The analytical method reliably identified 64 different drugs of abuse using a single extraction in oral fluid at forensically relevant cut-off concentrations. To demonstrate the method as fit for use, a pilot study was completed which compared the drug detection in 103 matched blood and oral fluid samples collected in suspected DUI investigations from the same individuals.

P-095

Evaluation of Blood and Oral Fluid Collection Methods: A Pilot Study of Over 100 Samples

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Abstract

Introduction: As oral fluid gains momentum as a viable alternative to blood in forensic toxicology, it is essential to evaluate its performance in real-world casework. This pilot study compares drug detection in 103 matched blood and oral fluid samples collected in suspected DUI investigations from the same individuals and then analyzed by two laboratories: one using blood testing protocols, the other applying recently validated oral fluid methods. Oral fluid testing involved immunoassay screening followed by qualitative confirmation via liquid chromatography–tandem mass spectrometry (LC-MS/MS). This study assesses the utility and practical application of oral fluid testing in forensic toxicology.

Objectives: To assess the concordance between matrices for commonly encountered drugs of abuse as well as explore the feasibility and limitations of implementing oral fluid as an alternative biological matrix in forensic toxicology casework.

Methods: A total of 103 matched case samples were collected in collaboration with the Louisiana State Police Crime Laboratory in Baton Rouge, which performed blood analyses for all cases. The corresponding oral fluid samples were analyzed by the North Louisiana Crime Lab in Shreveport. Oral fluid underwent initial screening via enzyme-linked immunosorbent assay (ELISA), with LC-MS/MS used for confirmation. Due to the ease of collection, oral fluid samples were collected, on average, 38 minutes before the corresponding blood draws. Oral fluid results were compared to blood toxicology findings to evaluate matrix agreement and overall detection performance.

Results: Data from the oral fluid immunoassay screening and LC-MS/MS confirmation were compared as well as the confirmation results to the blood results. Overall, strong agreement was observed between matrices. Seventeen cases in the pilot study demonstrated discrepancies between oral fluid and blood toxicology results, likely due to differences in analytical scope, cut-off concentrations, timing of sample collection, or pharmacokinetics. Of these, fifteen cases involved analytes detected in oral fluid but not in blood. The most frequently observed substances in this group included buprenorphine and THC (three cases each), followed by 6-MAM, cocaine, and methamphetamine/amphetamine (two cases each), and fentanyl, mitragynine, and tapentadol (one case each). Conversely, six cases showed analytes present in blood but not in oral fluid. Benzodiazepines—specifically oxazepam, temazepam, alprazolam, and clonazepam—were the most missed drug class in oral fluid. One case revealed THC (parent and metabolites) and trazodone in blood but not in oral fluid.

Discussion: The findings of this pilot study demonstrate strong overall concordance between oral fluid and blood testing for commonly encountered drugs of abuse in forensic casework. The high specificity observed in the oral fluid LC-MS/MS confirmatory method supports its reliability as an alternative matrix for qualitative drug detection, the biggest limitation in oral fluid being the benzodiazepine class of drugs. The results suggest that oral fluid testing can serve as a practical and less invasive alternative to blood in routine forensic toxicology investigations, particularly when timely and on-site collection is needed.

P-096

Validation of National Safety Council Tier 1 Scope of Analysis in Oral Fluid using Immunalysis ELISA kits on a Semi-Automated Tecan Freedom EVO 75

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Abstract

Introduction: Oral fluid has become an increasingly accepted alternative to blood in DUI investigations due to its ease of collection, observed sampling, and speed of collection time relative to the incident. However, its smaller sample volume and lower analyte concentrations necessitate highly sensitive and specific analytical methods. Immunoassay-based screening remains a practical first-line approach, particularly when integrated with semi-automated liquid-handling platforms to improve consistency and throughput. The Tecan Freedom EVO 75 is a compact, semi-automated system capable of executing complex pipetting protocols with minimal manual intervention, making it suitable for medium volume laboratories. This study aimed to validate a 15-drug panel (table 1) for presumptive screening of drugs of abuse in oral fluid using this semi-automated system. Method performance was evaluated according to ANSI/ASB Standard 036 guidelines, with the additional objective of aligning validation parameters with the National Safety Council's Tier One drug testing guidelines (2021 update) to ensure forensic and regulatory relevance.

Objectives: To validate a fifteen drug screening panel using a semi-automated Tecan Freedom EVO 75 instrument for presumptive drug testing in oral fluid.

Methods: Immunalysis provides separate 96-well microplates for each test, each coated with specific antibodies. The Tecan instrument pipettes diluted oral fluid into each well, along with 25 µL of THC preincubation buffer for the cannabinoid wells only. The cannabinoid plates are incubated at room temperature for one hour. Then, 100 µL of the appropriate conjugate is added to each well and incubated for an additional 60 minutes at room temperature. Wells are washed six times with 350 µL of deionized water. Subsequently, 100 µL of the appropriate tetramethylbenzidine (TMB) reagent is added to each well, followed by a 30-minute incubation at room temperature. The colorimetric reaction is stopped with 100 µL of 1N hydrochloric acid, and optical density is measured at 450 nm and 620 nm. Method validation evaluated parameters outlined in ANSI/ASB Standard 036, *Standard Practices for Method Validation in Forensic Toxicology*.

Results: Precision was assessed at three concentrations (50% of the cutoff, the cutoff, and 100% above the cutoff concentration) whereas each concentration was analyzed in quadruplicate over five batches. Intra- and inter-day precision coefficients of variation (CVs) ranged from 0.8% to 18.3%. Additionally, the grand mean ± two standard deviations of both the low and high concentration pools did not overlap with the grand mean of the decision point, supporting assay discrimination at the cutoff. Precision was also evaluated at equivalent concentrations for any analytes demonstrating cross-reactivity between 1–100%, as indicated in the manufacturer's insert, along with other common designer drugs not listed. The precision of analytes with cross reactivity produced acceptable results. The only exception was 7-aminoclonazepam, which had QCs overlap the cutoff. Lastly, carryover was assessed using the system's two-tip configuration, and no carryover was observed at 20× the cutoff concentration.

Discussion: The fifteen drug panel oral fluid immunoassay panel met all required acceptance criteria for precision at the cutoff, cross-reactivity, and carryover when implemented on the semi-automated Tecan Freedom EVO 75 platform. The method meets the performance criteria outlined in ANSI/ASB Standard 036, NSC recommended cutoffs for tier one testing, and is suitable for use as a presumptive screening tool in forensic toxicology. A LC/MS/MS confirmation method will be validated along with a pilot study of over 100 blood draws and oral fluid samples to demonstrate it is fit for purpose.

Table 1: Scope of Analysis

Analyte:	Limit of Detection:
Amphetamine	20 ng/mL
Benzodiazepines	5 ng/mL
Buprenorphine	1 ng/mL
Carisoprodol	500 ng/mL
Cocaine	15 ng/mL
Fentanyl	1 ng/mL
Ketamine	10 ng/mL
Methadone	20 ng/mL
Methamphetamine	20 ng/mL
Opiates	30 ng/mL
Oxycodone	30 ng/mL
PCP	2.5 ng/mL
Cannabinoids	4 ng/mL
Tramadol	50 ng/mL
Zolpidem	10 ng/mL

P-097

A Novel DESI MS-Based Screening Method for Ultra-Rapid Detection of Drugs Metabolites in Urine.

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Abstract

Introduction: Substance use disorder is a serious health issue in the United States. According to the CDC, an average of 224 daily overdose deaths were reported in 2022. Medication-Assisted Treatment (MAT), which combines counseling, behavioral therapies and FDA approved medications such as buprenorphine, has had a substantial beneficial impact. Drug testing is an important component of treatment, where it serves as a deterrent and enables compliance monitoring. Drug testing typically involves an initial immunoassay screen for the detection of classes of drugs, followed by mass spectrometry-based analysis for the definitive identification and quantitation of drug metabolites. Urine is a common specimen type. Although fast and low cost, immunoassay screens can yield false positive or false negative results due to non-specific cross-reactivity. Furthermore, the limited ability to detect novel psychoactive substances (NPS) renders immunoassay screens unsuitable in the current landscape of emerging designer drugs. Mass spectrometry-based analysis is sensitive, specific and flexible in its ability to detect NPSs. However, liquid chromatography (LC) contributes to extended turnaround times.

Objectives: The goal of this study was to develop an LC-free, ultra-rapid mass spectrometry-based screening method capable of routine detection of drug metabolites in urine.

Methods: Standards from 45 different drugs metabolites (licit and illicit) were used to spike drug-free pooled urine at concentrations ranging from 0.8 to 500 ng/mL. Specimens were analyzed as is or following solid phase extraction or dilution with isopropanol (IPA). Ultimately, samples (1ul) were spotted on PFTE-coated glass slides resulting in 0.8 to 500 pg of material within each sample spot. The spots were analyzed on a Waters™ Xevo™ G2 XS Mass Spectrometer equipped with an in-house built Desorption Electrospray Ionization (DESI) Autoloader source for high throughput plate reading. The MS and DESI parameters were optimized to the mass range of m/z 50-600 in positive mode and all sprayer and flow settings were optimized for highest sensitivity. A Waters SELECT SERIES™ Cyclic™ IMS equipped with a DESI™ XS source was used for compound validation and the separation of isobaric compounds. A targeted method using extracted ion chromatograms and MS/MS validation was developed to identify target drug metabolites.

Results: The time of measurement was 1-2 seconds/per sample resulting in less than 3 minutes per 96-well plate. Analysis of samples without dilution showed significant loss of signal, which was partially recovered upon dilution with IPA. Solid phase extraction delivered the best performance, which was further enhanced with use of a TQ Absolute MS analyzer. Quantitative analysis using analyte-matched internal standards produced acceptable linearity (R-squared > 0.99).

Discussion: In this study, we demonstrated that DESI MS-based analysis enables ultra-rapid detection of 45 drug metabolites in urine. While simple sample preparation approaches may be suitable for most compounds evaluated, solid phase extraction was necessary to detect all compounds, especially ones with low limit of quantitation. DESI MS-based analysis has the potential of becoming a screening method for robust detection of drug metabolites in urine.

P-098

Oops, I Injected Too Much! Smart Solutions for High Analyte Concentrations and Carryover in LC-MS Monitoring

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Abstract

Background/Introduction: In forensic toxicology laboratories, routine quantification of drugs of abuse (DoA) in biological samples using validated liquid chromatography–mass spectrometry (LC-MS) methods often encounters samples with analyte concentrations exceeding the upper limit of quantification. Such high concentrations can compromise the accuracy of quantification and increase the risk of carryover to subsequent injections. To mitigate these issues, it is essential for LC-MS data acquisition software to incorporate features that allow users to define thresholds, such as upper concentration limits, and evaluate each injection in real-time. This enables automated responses to prevent carryover and maintain the integrity and reliability of the dataset.

Objectives: This study aims to demonstrate the real-time intelligent features of Thermo Scientific™ TraceFinder™ and Chromeleon™ software in monitoring and managing samples with analyte concentrations exceeding user-defined thresholds when using Thermo Scientific LC-MS systems for the quantification of DoA in forensic samples.

Methods: A mixture of cocaethylene, oxazepam, methadone, methamphetamine, and PCP, along with their corresponding internal standards, was spiked into 20-fold diluted blank human urine at concentrations of 0.5, 5, 100, and 1000 ng/mL. A 1 µL mixture was directly injected onto a Thermo Scientific™ Vanquish™ Flex UHPLC system coupled to a Thermo Scientific™ Orbitrap Exploris™ 120 mass spectrometer equipped with the OptaMax NG HESI source. Analyte separation was achieved on a Thermo Scientific™ Accucore™ Biphenyl column at 0.5 mL/min flowrate with 0.1% formic acid in water as the mobile phase A and 0.1% formic acid in methanol as the mobile phase B. The calibration curves were generated using the samples with concentration 0.5, 5, and 100 ng/mL, and the sample with the concentration 1000 ng/mL was used to trigger the pre-defined Carryover Limit of 500 ng/mL in Intelligent Sequencing (Intel Seq) in TraceFinder and System Suitability and Intelligent Run Control (SST/IRC) in Chromeleon. In Intel Seq, the Failure Action of Inject Auto Sample of up to 3 strong wash samples and Continue was tested. Similarly, three Custom Injection Variables in SST/IRC were setup and tested independently: injection.inject_volume and “AutoDilution” determined based on range above calibration, and inject solvent blank with a column wash method.

Results: Both Intel Seq and SST/IRC provide intelligent features that monitor various sample types across different test cases. These include the presence of analytes in blank matrix workup, whether analyte concentrations in quality control samples meet expected values, and most importantly, whether a sample with unknown analyte concentration exceeds a certain value that may cause unreliable quantification results or potential carryover. In Intel Seq within TraceFinder, users can define a "Carryover Limit" for each or selected compounds and choose appropriate intervention actions ("Failure Actions") from options such as "Continue," "Stop," "Reinject," "Inject Auto Sample" (blank matrix sample, pre-defined solvent blank, or strong wash sample), or "Inject Auto Sample and Reinject" with a maximum action count. We selected 500 ng/mL as the Carryover Limit and triggered Inject Auto Sample up to 3 strong wash samples, ensuring that the flow path was thoroughly washed

and no significant levels of analytes remained. SST/IRC in Chromeleon offers additional Failure Actions via user-defined "Custom Injection Variables," and we tested and triggered smaller injection volumes (1/4 of the original volume), "AutoDilution" with a 4x dilution factor in the sample loop, and injection of a solvent blank with a different instrument method to efficiently wash the flow path.

Conclusion/Discussion: This study successfully demonstrated the setup and utilization of intelligent features in both TraceFinder and Chromeleon software to monitor analyte quantification results in real-time. The software allows users to select appropriate actions to adapt to their specific needs, ensuring reliable quantification and preventing carryover in forensic toxicology applications.

Conflict of Interest

Salary from Thermo Fisher Scientific

P-099

Autopsy and Toxicologic Approach to Suspected Laundry Detergent Exposure.

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Abstract

Introduction: A 36-year-old male with a past medical history of alcoholism, chronic pancreatitis, and housing instability was found by family members deceased in his vehicle. At the scene was an opened container of liquid laundry detergent as well as bloody residue on the container and in the vehicle. Exposures to liquid and encapsulated laundry detergent have become a major concern for public health officials, particularly in young children. The ease of access and colorful appearance of encapsulated detergents makes them appealing to children, while social media have popularized its ingestion in adolescents and young adults.

Objectives: The objective is to provide diagnostic tools to approach cases in which exposure to laundry detergent may be involved in the death of a decedent. Diagnostic tools include use of medical records, physical examination, autopsy findings, and toxicology findings.

Methods: This is a case study. Review of the literature was performed to identify existing methods to recognize signs and symptoms of laundry detergent exposure.

Results: Laundry detergents have various ingredients that can cause different autopsy findings, such as oxygenated bleaches, fragrance, surfactants, ethanol, and propylene glycol. Autopsy examination demonstrated frothy edema within the lungs, no esophageal erosions, and floral-scented, foamy gastric contents. Autopsies of ingestion demonstrate similarities to ingestion of other caustic substances, though detergents can be distinguished by their fragrance or frothy appearance upon agitation. Edematous organs, erosions, eschar, and erythema are common observations throughout the digestive tract in cases where ingestion is suspected. Toxicology workup included detection of propylene glycol and metabolic acidosis in the blood; ethanol in vitreous humor; and oil red staining for different tissues.

Discussion: Few deaths have occurred because of dermal, ocular, or oral exposures, though hospitalizations occur frequently. However, there is a rise in ingestion of laundry detergent, making it a public health concern since the 2017 social media popularization. Within the first several weeks of 2018, 86 cases of laundry detergent ingestion were reported by the American Association of Poison Control Centers. Available toxicology studies are limited in their ability to identify key ingredients in laundry detergents, thus toxicologists and medical examiners will need to use various tools to identify signs that suggest exposure to detergents.

P-100

Simultaneous Identification and Quantitation of NPS in Human Whole Blood Using QTRAP Technology

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Abstract

Introduction: The emergence and rising number of novel psychoactive substances (NPS) on the recreational drug market continues to pose health and safety challenges worldwide. These substances vary greatly in potency and purity, and thus often require only a small amount to cause acute intoxication. NPS analysis is challenging for forensic toxicology laboratories due to their structural diversity and complexity. A robust screening workflow must be comprehensive, sensitive and possess a high degree of selectivity to minimize interferences from biological matrices. Further, large NPS panels are difficult due to the presence of multiple isobaric compounds, requiring chromatographic separation to avoid false positive detections. Screening workflows must be adaptable to meet the constant discovery of new NPS compounds.

Objectives: In this study, a comprehensive targeted drug screening method was developed for the quantitation of 130 NPS in human whole blood. In addition to multiple reaction monitoring (MRM) acquisition, the method included the acquisition of enhanced product ion (EPI) scans using the QTRAP technology of the SCIEX 5500+ system. The MS/MS spectra collected during the EPI scans allowed for increased compound detection confidence through MS/MS library matching against a user-generated MS/MS database.

Methods: The selected panel of 130 NPS includes 22 stimulants, 13 benzodiazepines, 6 dissociatives, 16 hallucinogens, 45 synthetic opioids and 28 synthetic cannabinoids. The analyte list was developed with the assistance of the Center for Forensic Science Research and Education (CFSRE) and was curated based on prevalent NPS that have emerged on the recreational drug market over the past 2-3 years. NPS were extracted from human whole blood using a protein precipitation procedure. Liquid chromatography was performed using a SCIEX ExionLC AC and mass spectrometry analysis was performed on a SCIEX QTRAP 5500+ system where MS and MS/MS data were acquired using positive electrospray ionization. Experimental MS/MS spectra were compared against a custom-built library that was previously generated from the individual neat standard mixtures. The experiments were designed to establish proof of concept rather than full validation.

Results: A series of spiked calibrator blood samples ranging from 0.1 to 50 ng/mL were injected in triplicate to evaluate the quantitation and qualitative performance of the method. The scheduled MRM method enabled quantitation of the 130 NPS down to the lowest (0.1 ng/mL) calibrator level with acceptable precision and accuracy. The MRM-IDA-EPI method provided an added level of confidence for the identification of structurally related analytes eluting off the column at similar retention times using spectral library database searching. In addition, linearity was observed across the concentration ranges analyzed with R^2 values greater than 0.99 for all 130 NPS.

Discussion: A targeted drug screening method using MRM survey scans followed by data dependent triggering of full scan MS/MS was developed for fast, selective and sensitive detection and identification of a panel of 130 NPS extracted from human whole blood. The results demonstrate that the combination of this high-quality quantitation data with the library

matching has the capability of significantly reducing the rate of the false positive and false negative results that are commonly seen with other screening methods. Specifically, the method chromatographically separated 24 out of 29 isobaric NPS groups, comprising a total of 70 compounds. Further, 2 isobaric groups contained compound with unique fragments.

P-101

A Fully Customizable Workflow for The Resubmitting of Samples Using Customized Rules Without User Intervention

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Abstract

Introduction: The complexity and rigorous nature of forensic toxicology analysis often necessitates the re-analysis of samples to ensure rigorous and reliable results. This re-analysis can be due to various reasons such as sample saturation/overload, unresolved isobaric compounds, or the need for a confirmation on a different instrument. Currently, this process is mostly manual, involving a user processing the first batch of data and subsequently resubmitting selected samples.

Objectives: Recently, a feature was included in the vendor software that enabled the automatic production of a report - this project aims to leverage this feature to automate the resubmission of samples, thereby compressing the time required for completing the analysis.

Methods: The project leveraged automatic reporting (SCIEX OS version 3.4.5) and Control Application Programming Interface (API v12) software from SCIEX. Using this reporting feature enabled the construction of a pre-formed batch template without user intervention and this, in turn, is converted into a batch and submitted to the instrument. All steps were performed without user involvement.

Results:

We set out to identify a number of use cases for an automated re-submission workflow. Through several discussions, 3 main workflows were identified as high priority for laboratories performing drugs of abuse analyses. In order of complexity, these are:

- Variable Injection Volume. If the peak is too large, the sample is re-injected using a lower injection volume.
- Separate Isobaric Species. If a positive peak is identified, a second method ("Method B") is run to separate isobaric species for that compound using a longer separation or requiring a different column.
- Screen on Instrument 1 and Confirm on Instrument 2. In this scenario, a screening method is performed on "Instrument 1" and suspect samples automatically run on "Instrument 2" as a confirmation.

For each scenario, efficiencies are found that are in direct proportion to the number of repeat injections a lab routinely needs to perform. The exact time savings for each is ultimately dependent on how many samples need to be injected. For example, if 20 samples need to be reinjected then a time savings of at least 20x the runtime would be expected. Further, if a sample needs to be reinjected after batch processing, presumably during the morning after an overnight run, there is the additional delay when equilibrating the instrument and then adding to the sample queue for the day.

Discussion: This project demonstrated the design and implementation of a fully customizable workflow for unattended resubmission of samples using flexible rules. By automating the decision-making process, the time required for completing toxicology analyses (defined as sample receipt to final report) can be significantly reduced, enhancing efficiency and accuracy in toxicology workflows.

P-102

Multi-Phase Validation of LC/QTOF-MS as a Screening Technique for Blood and Oral Fluid

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Abstract

Introduction: Enzyme immunoassay (EIA) technology is the primary screening technique for the Toxicology Discipline of the Alabama Department of Forensic Sciences (ADFS). EIA provides rapid screening but lacks specificity and ability to detect novel psychoactive substances. ADFS has expanded screening capabilities to include liquid chromatography paired with quadrupole-time-of-flight mass spectrometry (LC/QTOF-MS). Screening of blood and oral fluid specimens via LC/QTOF-MS has been validated for 63 analytes, to include fentanyl, xylazine, mitragynine, and other drugs commonly detected in toxicology cases at ADFS. Of these 63 analytes, 30% are not cross-reactive with targets of the EIA screen used at ADFS. The LC/QTOF-MS will be primarily used as an expanded screen for suspicious cases that screen negative using EIA.

Objectives: To discuss the validation of LC/QTOF-MS instrumentation as a screening technique for blood and oral fluid specimens.

Methods: Blood and oral fluid specimens were extracted via protein crash with -20°C acetonitrile. Samples are filtered through 0.2 µm Thomson filter vials, dried down with nitrogen gas, and reconstituted with a mixture of 95% mobile phase A–water with 5mM ammonium formate with 0.1% formic acid–and 5% mobile phase B–acetonitrile with 0.1% formic acid. Samples were analyzed with an Agilent 1290 Infinity II Binary Liquid Chromatograph coupled with an Agilent 6545 QTOF in TOF only mode. Analytical separation was achieved with an Agilent Poroshell 120 EC-C18 (2.1x100mm, 2.7µ) column held at 55°C. Results were analyzed using Agilent MassHunter software. Compounds were identified through comparison to a Personal Compound Database and Library (PCDL) containing retention times, molecular weights, and chemical formulas. Validation acceptance criteria included adequate peak shape, mass accuracy of ≤ 20 ppm, retention time difference of ≤ 0.25 minutes between the observed and PCDL retention times, and a score of ≥ 85.

Validation of analytes on the LC/QTOF-MS occurred in three phases. Phase one included common drugs of abuse, such as cocaine, fentanyl, and methamphetamine. Phase two included designer benzodiazepines and xylazine. Phase three included pharmaceuticals such as citalopram, hydroxyzine, and pregabalin. Validation activities were guided by ANSI/ASB Standard 036 and included studies assessing limit of detection (LOD), interferences, carryover, ion suppression, stability, and previously analyzed casework. Case studies were selected to showcase the applications of this method for both blood and oral fluid case submissions.

Results: Phase one resulted in the successful validation of 20 target analytes and 14 deuterated internal standards. An additional 25 analytes were added to the method's scope of analysis during phase two, and 18 analytes were added during phase three. Norsertraline was removed from the method's scope, as its LOD exceeded toxicologically relevant concentrations in both matrices.

LODs of validated analytes were deemed appropriate for DUI testing, ranging from 2 to 100 ng/mL in blood and 2 to 2,000 ng/mL in oral fluid. Minimal matrix and analyte interferences were observed. Stability results demonstrated 84% of analytes are stable in both matrices for at least

24 hours after extraction. Analyte carryover was not observed at concentrations below 500 ng/mL.

Discussion: The LC/QTOF-MS has been successfully validated for 63 target analytes and 14 deuterated internal standards in blood and oral fluid. In 108 instances of validated analytes being previously reported at a concentration above the LC/QTOF-MS LOD, validated analytes met acceptance criteria for the LC/QTOF-MS in 93% of instances. In addition, the inclusion of LC/QTOF-MS in casework led to positive identification of compounds not cross-reactive with targets of the EIA screen used at ADFS in a total of 41 cases. This method provides laboratories with an option for a non-EIA screen or a supplement screening method for blood and oral fluid specimens. Additionally, a multi-phase validation approach provides a framework for systematically adding targets to a method over time, mitigating some of the challenges associated with the validation process.

P-103

Recent Cases Involving an Uncommon Synthetic Stimulant: α-PVP

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Abstract

Introduction: α-Pyrrolidinovalerophenone (α-PVP), also known as Flakka, is a psychoactive stimulant structurally related to pyrovalerone and Methylenedioxypyrovalerone (MDPV) that has been used as a recreational drug. Like other synthetic stimulants, it produces effects such as euphoria, excitement, and alertness, but adverse psychological effects such as hallucinations, confusion, paranoia, and aggression have been reported. It made its appearance on the market in early 2010s seemingly peaking in popularity between 2014 and 2016. Flakka was alluring as it was easily accessible as a designer drug on the internet, either on its own or in combination with other drugs and sold recreationally as “ecstasy” or “bath salts.” Since its emergence on the drug user market, α-PVP has been encountered in toxicological casework and was temporarily scheduled by the DEA in 2014. Prevalence had decreased after permanent federal scheduling in 2017, and other synthetic stimulants gained popularity. At NMS Labs, detection of α-PVP in toxicological casework decreased notably from the end of 2018 to midyear 2021, however, recent data suggests a possible resurgence in α-PVP use.

Objectives: This study examines recent detections of α-PVP in toxicological casework with case history discussion.

Methods: Data were extracted from NMS Labs’ LIMS system for all blood cases that reported a positive result for α -PVP from January 2015 to June 2025. Submitted casework originated primarily from medical examiner or coroner agencies for postmortem death investigation. Other submissions from law enforcement involved blood taken from living individuals suspected of impaired driving.

Results: In the time-period studied, 246 blood cases were confirmed positive for α-PVP. Samples were tested using a liquid-liquid extraction technique followed by LC-MS/MS quantitation using a fully validated method. Approximately 230 (88%) cases were reported prior to 2019, with the majority of those (63%) reported in 2015. No blood cases were reported positive from November 2018 until August 2021.

Approximately 80% of results were detected in postmortem blood with concentrations ranging from 2.0 to 3900 ng/mL (n=202). α-PVP concentrations in impaired driving casework ranged from 5.3 to 350 ng/mL (n=44).

Since August 2021, 15 cases have confirmed α-PVP in postmortem blood specimens, the majority recently. α-PVP has not been detected in impaired driving casework since 2018. From July 2024- June 2025, NMS Labs has confirmed α-PVP in 10 postmortem blood samples involving submissions from three states; Florida, Utah and Idaho. Polypharmacy was common. One case involved the death of a 1-month-old child found unresponsive in a sleeping area; α-PVP was the only positive finding in the case.

Discussion: α-PVP, an uncommon finding since 2018, has observed a recent, albeit slight increase in detection. Navigating the landscape of novel psychoactive substances presents a challenge to toxicology labs, as developing and maintaining the necessary analytical methods is an ever-evolving

task. This data suggests that its inclusion in the analytical scope may be worthwhile to ensure appropriate reporting of all relevant toxicological findings.

P-104

Let's Test That: A Novel, Single Instrument Setup for Compliance with the ANSI/ASB 120 Standard for Impaired Driving Investigations.

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Abstract

Introduction: Over the past few years there has been an onus on forensic laboratories responsible for impaired driving investigations to adopt a more robust testing protocol. The ANSI/ASB 120 standard which was introduced in 2021 outlines the specific analytes and corresponding concentration "cutoffs" that should be tested for in regard to impaired driving investigations. This standard comprises the combined efforts of the forensic toxicology community and is being implemented as standard practice in many forensic laboratories. However, the ability for laboratories to adhere to this standard is becoming more challenging due to constrained budgets and increased cost of instrumentation.

Objectives: The objective of this study is to develop a robust, single instrument setup that allows for the quantitative analysis of ethanol via GC/FID dual column, dual FID and a qualitative drug screen via GC/MS/MS for the drug analytes listed out in the ANSI/ASB 120 standard.

Methods:

- **Sample preparation** – The ethanol analysis utilized a volatile component certified reference material (CRM) from cerilliant and an N-propanol (1%) in water internal standard was added to each sample. The CRM samples consisted of aqueous ethanol calibrators ranging from 0.01 g/dL to 0.50 g/dL, and a multi-component standard containing ethanol, acetone, isopropanol, and methanol to attain proper retention times of those analytes. A whole blood matrix blank and control samples were also run to verify accuracy. Each sample consisted of 100 uL of sample and 600 uL of the internal standard. The GC/MS/MS drug screen utilized a CRM of each analyte, and 5 deuterated internal standards. The samples were spiked to match the concentration of the lower limit of detection for each of the 32 analytes listed in the ANSI/ASB 120 standard. These samples were extracted using a two-step Quechers extraction technique and evaporated to dryness and reconstituted in 50ul of BSTFA-1% TCMS and 50 ul of methanol and heated at 70^{0}C for 30 minutes, cooled and 1uL injected onto the instrument. The methods utilized a Thermo Scientific™ TriPlus™ RSH SMART Autosampler for headspace and liquid injections and injected the samples into a Thermo Scientific™ TRACE™ 1610 GC.
- **Chromatography** – Three different GC columns in a single oven were used. The headspace GC/FID analysis used a TG-624 Sil MS column (30 m x 0.25mm ID x 1.4um) and a Trace TR-WAX column (30m x 0.25mmID x 1.0 um) with each being tied to an individual FID detector. The GC/MS/MS drug screen used a TG-5 Sil MS column (30m x 0.25mmID x 0.25 um) with the sample being analyzed on a Thermo Scientific™ TSQ™ 9610 Triple Quadrupole GC/MS/MS.

Results: The dual column, dual FID method was able to readily detect and identify all of the volatile analytes and quantitate ethanol in the headspace method. The headspace method provided R-squared values on the calibration curve for ethanol above 0.99. The MS/MS method

showed great sensitivity as it was able to detect and identify all 32 drug analytes listed in the ANSI/ASB 120 standard at their LOD concentrations which vary for each analyte and range from 0.5 ng/mL up to 1000 ng/mL.

Discussion: The single instrument setup proved to be able to accurately and efficiently complete the process of quantitating ethanol values while also allowing for a targeted initial drug screen of forensic samples submitted for impaired driving investigations on a single GC/MS/MS instrument with FID detectors. Hopefully this will allow more laboratories the opportunity to more efficiently test their suspected impaired driving samples in accordance with the ASB 120 standard.

Conflict of Interest

I am an employee of Thermo Fisher Scientific.

P-105

Automated Solid Phase Extraction Using MicroElution Plates for Fentanyl Analogs, Nitazenes, and Xylazine in Human Urine

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Abstract

Introduction: The continued prevalence of fentanyl and its analogues, as well as nitazenes and xylazine, underscores the urgent need for reliable analytical tools in clinical and forensic toxicology. Accurate detection of these substances in biological matrices is critical in clinical and forensic toxicology. Liquid chromatography-tandem mass spectrometry (LC-MS/MS) offers high sensitivity and selectivity, but achieving reproducible results at high-throughput requires optimized sample preparation. This presentation highlights the use of a microplate based SPE format offering micro-elution capabilities for the extraction of a panel of 30 analytes from human urine using automated workflows.

Objectives: The adoption of micro-elution plate SPE enables streamlined automation, improved sample throughput, and significantly lower elution volumes compared to traditional SPE columns. This study demonstrates extraction workflows utilizing the Tecan Resolvex® A200 for a semi-automated method and a fully automated workflow utilizing the Tecan Resolvex® i300 integrated on the Fluent® platform. The Resolvex® systems automate the SPE steps for sample loading, washing, and elution, resulting in efficient sample processing workflows.

Methods: A panel of standards was spiked into drug-free human urine (Utak) yielding a concentration of 1 ng/mL. This concentration sits near typical clinical toxicology decision points for fentanyl analogs, nitazenes, and xylazine. The aim was to demonstrate automated SPE feasibility and consistency at a low, analytically challenging level.

Sample panel:

(+/-)-cis-3-Methylfentanyl, (+/-)-beta-Hydroxythiofentanyl, 2-Thiofuranyl fentanyl, 4-hydroxy Xylazine, Acetyl norfentanyl oxalate, Acryl fentanyl, Benzoyl fentanyl, Butyryl fentanyl, Carfentanil oxalate, Cyclopropyl fentanyl, Etonitazene, Fentanyl, Furanyl fentanyl, Isobutyryl fentanyl, Isotonitazene, Methoxyacetyl fentany, Metodesnitazene, Norcarfentanil oxalate, Norfentanyl oxalate, Ocfentanil, o-Fluorofentanyl, ortho-Methylacetyl fentanyl, para-Fluorobutyryl fentany_ PFBF, Remifentanil, Sufentanil Citrate, Tetrahydrofuranyl fentanyl, Tetramethylcyclopropyl fentanyl, U-48800, Valeryl fentanyl, Xylazine.

SPE was performed using Tecan's HPSCX sorbent in a 96-well microplate format. HPSCX is a mixed mode sorbent providing reverse phase and strong cationic exchange chemistry. Each well of the plate contains 3mg of 30um sorbent. Low elution volumes were used, totaling 50uL, delivered as two 25uL dispense and elute steps. For each of the SPE workflows, the Resolvex® A200 and i300 instruments were used for positive pressure loading and solvent dispensing, while the Resolvex® i300 also provided drying of purified samples. Resuspended samples were analyzed using the Sciex 5500 QTrap MS platform. Direct injection of diluted eluates without drying was also explored. Both approaches produced comparable results for the most analytes, with direct injection offering time savings and reduced sample handling.

Results: The micro-elution plate SPE workflow was evaluated for extraction efficiency and reproducibility. LC-MS/MS analysis of the extracted samples provided ample sensitivity for most of the compounds, at a concentration of 1ng/mL in urine. To assess extraction efficiency, the recovery of samples was calculated using a post-extraction spike in. The recovery ranged from approximately 67 - 100%, with the majority of analytes showing recoveries ≥90%. Some analytes had recoveries less than 80%, such as 4-hydroxy Xylazine at 67% and acetyl norfentanyl oxalate at 74%. Reproducibility was assessed with five technical replicates (repeated extractions and analyses of the same spiked urine under identical SPE conditions). Low CVs confirmed high intra-assay precision and quantitative stability. Two-thirds of analytes had CVs below 10%, with an overall range of 3-25%, with outliers being Benzoyl fentanyl at 12%, o-Fluorofentanyl at 11%, para-Fluorobutyryl fentanyle PFBF at 25% and Tetrahydrofuranyl fentanyl at 14%.

Discussion: The SPE methods have been proven to extract a sample panel consisting of fentanyl and its analogues, nitazenes and xylazines from human urine. By utilizing the Tecan Resolvex® instruments and the micro-elution plate SPE workflow, the automated method enabled low-volume elution, reduced hands-on time, and an efficient protocol for high-throughput toxicology assays and testing.

P-106

Development and Validation of an Enzyme Immunoassay Screen and Achiral/Chiral LC-MS/MS Confirmation Methods for Ketamine Analysis in Hair

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Abstract

Introduction: Ketamine has legitimate medical use in the clinic as an analgesic, with an additional recent approval of the single ketamine isomer, (*S*)-ketamine/esketamine, as a therapy for treatment resistant depression. Unfortunately, ketamine is also prone to non-medical use, often as a club or party drug. Herein, we describe development and validation of a ketamine enzyme immunoassay screen, and both achiral and chiral LC-MS/MS confirmation methods. The chiral LC-MS/MS confirmation method offers a potential methodology to determine if a subject ingested (*S*)-ketamine/esketamine solely as part of a prescription treatment resistant therapy regimen.

Objectives: To develop an immunoassay screen and chiral/achiral LC-MS/MS confirmation methodology to detect ketamine and norketamine in hair.

Methods: Samples were screened by (1) drug extraction from hair using a patented reductive digest (United States Patent 8,084,215) and (2) performing an in-house developed microtiter plate immunoassay using a polyclonal antibody with a ketamine cutoff established at 500 pg/mg hair. The confirmation process consisted of a new hair aliquot that was first washed using an extended 90% ethanol wash, followed by hair drug extraction with acidic methanol, solid phase clean-up and confirmation using an AB SciEX 3200 LC-MS/MS in positive multiple reaction mode at cutoffs of 500 pg ketamine and 50 pg norketamine/mg hair. Two LC-MS/MS confirmation methods were developed, an achiral method to analyze ketamine and norketamine, along with a chiral method to separate and analyze ratios of (*R*) and (*S*)-ketamine isomers.

Results: The immmunoassay screen and LC-MS/MS confirmation methods were validated according to ANSI/ASB Standard 036. Immunoassay inter-assay precision was evaluated at -100%, -75%, -50%, -25%, 500 pg ketamine/mg hair cutoff calibrator, +25%, +50%, +75% and +100% of the cutoff calibrator concentration. The immunoassay inter-assay precision CV was less than 6% at all concentrations. The ranges of two standard deviations of the immunoassay B/B_0 values for the -50% and +100% controls did not overlap the grand mean B/B_0 of the cutoff concentration. Norketamine was a cross reactor at 25% of the 500 pg ketamine/mg hair immunoassay calibrator. Authentic hair samples containing ketamine and norketamine from the United States workplace testing population, composed of predominately pre-employment drug testing, were identified by the immunoassay screen at the 500 pg ketamine/mg hair cutoff. For all LC-MS/MS confirmation methods, the limit of detection (LOD) and limit of quantitation (LOQ) for the measured analytes were set at 10 pg analyte/mg hair, and the upper limit of linearity (ULOL) was set at 5,000 pg analyte/mg hair. Relative to the cutoff concentration of 500 pg ketamine and 50 pg norketamine/mg hair: intra-assay LC-MS/MS precision was assessed at -50%, -25%, cutoff, +25% and +50% of the cutoff concentration, with inter-assay LC-MS/MS precision evaluated at -50%, cutoff and +50% of the cutoff concentration over a period of five days, with acceptance criteria set at less than +/-20% CV for all measurement points for intra and inter-assay precision. Bias relative to the 500 pg ketamine and 50 pg norketamine/mg hair cutoff was assessed at -50%,

cutoff and +50% of the cutoff concentration over a period of five days, with acceptance criteria set at less than +/-20% at each measurement point.

Discussion: This work provides a validated methodology to rapidly screen for ketamine in hair by immunoassay, followed by LC-MS/MS confirmation after an extended 90% ethanol wash. Addition of a chiral LC-MS/MS confirmation method to separate (*R*) and (*S*)-ketamine provides a definitive method to adjudicate samples from subjects undergoing therapy for treatment resistant depression. Application of the chiral LC-MS/MS confirmation method towards the analysis of authentic samples containing ketamine from the United States workplace testing population revealed an approximately 1:1 mixture of (*R*) and (*S*)-ketamine isomers, indicating a source of ingestion other than only esketamine for this sample population.

Conflict of Interest

Neil Stowe and Ryan Paulsen are both employees and stockholders of Psychemedics Corporation.

P-107

Comparative Analysis of Urine and Oral Fluid Drug Testing: A 10,554-Specimen Study from Abbott Santa Rosa

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Abstract

Introduction: Urine remains the predominant specimen type for court-ordered drug testing due to the long detection windows (when compared to blood and oral fluid) and the availability of large testing menus. However, oral fluid testing offers a less intrusive, trauma-informed collection procedure. In U.S. drug courts individuals are often tested 2-3 times per week through observed collection of urine, oral fluid, or both. This study evaluates the concordance between urine and oral fluid drug testing results collected from the same individuals on the same day and analyzed for the same drug/metabolite(s) or class of drug.

Objectives: To assess the agreement between urine and oral fluid drug testing results, 10,554 paired specimens were screened for amphetamines, buprenorphine, benzodiazepines, cocaine, fentanyl, methadone, opiates, and/or THC at Abbott Santa Rosa in 2024. A subset of 2,768 pairs underwent confirmatory testing.

Methods: All procedures adhered to ASB 036 validation guidelines. Oral fluid was collected using Quantisal® devices. Screening was performed via Enzyme Immunoassay (EIA) on Beckman AU5800 analyzers using Microgenics or Immunalysis reagents. Confirmatory testing employed LC-MS/MS (Sciex 3200, 4000, or 6500). Cutoffs varied by matrix and analyte, with oral fluid generally requiring lower thresholds for detection.

SCREEN	Oral Cutoff (ng/mL)	Urine Cutoff (ng/mL)	Urine Pos Oral Neg	Urine Neg Oral Neg	Urine Pos Oral Neg	Urine Neg Oral Pos
Amp/Methamp	50	500/1000	873	8616	161	885
Buprenorphine	5	5	17	705	15	3
Benzodiazepines	20	200	110	10060	235	87
Cocaine	20	150/300	130	10248	50	104
Fentanyl	1	5	48	2513	153	9
Methadone	50	100	128	413	8	3
Opiates	40	300	76	10294	144	11
THC	4	20/50	1825	7500	684	417

CONFIRMATION	Oral Cutoff (ng/mL)	Urine Cutoff (ng/mL)	Urine Pos Oral Neg	Urine Neg Oral Neg	Urine Pos Oral Neg	Urine Neg Oral Pos
Amphetamine	15	250	844	33	14	23
Buprenorphine	1	0.5	16	0	5	1
Benzodiazepines	0.5	50	91	5	2	4
Cocaine	4	100	123	2	3	3
Fentanyl	1	0.5	46	13	5	5
Methadone	10	100	48	0	1	1
Opiates	8	100	75	3	2	2
THC	1	5	1728	7	76	6
Oxycodone	8	50	37	2	1	0

Results:

Screening Agreement: 94.7% overall concordance

- 2.6% urine-positive / oral fluid-negative
- 2.7% urine-negative / oral fluid-positive

Confirmation Agreement: 95.0% overall concordance

- 3.3% urine-positive / oral fluid-negative
- 2.7% urine-negative / oral fluid-positive

Conclusions: The high level of agreement between urine and oral fluid drug testing supports the viability of oral fluid as a reliable alternative matrix for drug screening and confirmation in court-ordered testing programs where individuals are tested frequently (2-3 times per week). These findings may inform policy and practice in forensic and clinical toxicology, particularly in contexts where trauma-informed collection is preferred.

P-108

An Eight-Year Retrospective Analysis of Carfentanil Presence in Nine Michigan Counties

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Abstract

Introduction: Data collected over an eight-year period as part of an opioid surveillance program were examined to assess the prevalence of carfentanil in nine surveyed counties in Michigan. Carfentanil is a Schedule II drug and is a synthetic opioid originally synthesized for veterinary medical use for the sedation of large animals. It is a fentanyl analog and is purported to be 100 times more potent than fentanyl. Carfentanil is not approved for human use; even trace amounts can lead to many adverse effects, including death. In 2018, Western Michigan University Homer Stryker M.D. School of Medicine (WMed) detected carfentanil through a managed opioid surveillance program, Swift Toxicology of Opioid Related Mortalities (STORM). STORM was initiated in September 2017 and aims to improve toxicological testing for overdose deaths in Michigan and to provide more rapid and accurate estimates of opioid-related fatalities and identify the specific opioids involved. After lying dormant for several years, carfentanil detection in Michigan began to reappear and rise in 2024 and 2025. Through STORM, fourteen carfentanil cases across nine Michigan counties from 2018 to 2025 have been identified.

Objectives: This study examines the prevalence and profile of carfentanil-related mortalities over eight years across nine Michigan counties using results generated via STORM.

Methods: In this study, we retrospectively reviewed postmortem data collected using an interactive dashboard (i.e., STORM Dashboard). Sample collection material is shipped from WMed to counties across Michigan. Currently, 53 out of 83 counties in Michigan send postmortem specimens collected at medical examiners' offices to WMed. Samples were analyzed using enzyme-linked immunosorbent assay specific for fentanyl; the cross-reactivity of this assay to carfentanil was less than 1%; however, all the carfentanil-positive samples were also positive for fentanyl. A solid-phase extraction technique for analysis by liquid chromatography-tandem mass spectrometry (LC-MS/MS) having a limit of detection of 1 ng/mL was also performed.

Results: The STORM dashboard revealed that fourteen carfentanil-related deaths were identified in the following nine Michigan counties: Eaton, Muskegon, Ingham, Livingston, Wayne, Genesee, Kalamazoo, Shiawassee, and Ionia. In 2018 and 2019, one case of carfentanil was identified each year. From 2020 to 2023, carfentanil appeared dormant and then reappeared in 2024, appearing in three cases. From January 2025 to May 2025, there were six reported cases of carfentanil-related mortalities, suggesting a possible resurgence. Of these fourteen cases, 79% were males and 21% were females, which is consistent with drug overdose trends in the U.S. Within the STORM data, key variables are highlighted, such as race, gender, location of death, date of death, and co-occurring drugs.

Discussion: The upward trend in carfentanil cases suggests that there has been a change in the illicit drug market and distribution patterns in Michigan over the recent years. A similar increasing trend in the presence of carfentanil in confiscated drugs has been reported by multiple Midwest law enforcement agencies. This renewed presence of carfentanil emphasizes the critical need for

toxicologists to continue to monitor drug trends. Without monitoring drug trends, it is difficult for toxicologists, health professionals, and public health experts to truly understand the impact drugs may have on the population and when drugs become dormant or reemerge.

P-109

BTMPS in the Illicit Opioid Supply: Emerging Trends and Analytical and Interpretive Challenges

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CFSRE, Horsham, PA, USA

Abstract

Introduction: BTMPS, also referred to as bis(2,2,6,6-tetramethyl-4-piperidyl)sebacate or Tinuvin 770, is an industrial chemical that began appearing in June 2024 in the recreational opioid supply as an adulterant accompanied by fentanyl. BTMPS is used as a light stabilizer and was originally evaluated for use in plastic materials. Early on, the presence of BTMPS in the drug supply was perplexing to forensic scientists and chemists. Some speculated its use could be related to perceived enhancement of pharmacological effects of fentanyl or more simply as a cutting or bulking agent. The chemical structure of BTMPS is dissimilar from most forensically relevant drugs; however, it contains substituted piperidine rings — a similar core moiety to fentanyl. In early to mid–2025, forensic laboratories began detecting substances with chemical linkages to BTMPS first in seized drug samples, and subsequently in toxicology casework, including tetramethyl-4-piperidinol, and soon after substances with ties to tetramethyl fentanyl, tetramethyl 4-AP and tetramethyl norfentanyl variants. The emergence of these substances and relevant intelligence information indicate BTMPS is likely linked to the manufacture of fentanyl-related precursors, byproducts, and reaction intermediates.

Objectives: This presentation provides an update for forensic toxicologists on new information surrounding the emergent adulterant BTMPS and newly discovered fentanyl-related substances.

Methods: Seized drug analysis was performed by gas chromatography mass spectrometry (GC-MS) and liquid chromatography quadrupole time-of-flight mass spectrometry (LC-QTOF-MS). Forensic toxicology testing was performed by LC-QTOF-MS. Resulting datafiles were stored for future data-mining purposes and the opportunity to conduct unknown analysis and structural elucidation. In addition, we examined data warehoused in the Drug Enforcement Administration (DEA)'s National Forensic Laboratory Information System (NFLIS) and partnered with the DEA's Special Testing and Research Laboratory for synthesis and confirmation of the substances identified.

Results: BTMPS has been detected in all regions across the United States and has been reported in every state except Wyoming (at the time of submission). The spread of BTMPS has been rapid, although the prevalence of the substances in seized drug samples has fluctuated over the last year. BTMPS has appeared in more than 600 drug materials tested by the CFSRE alone. In early 2025, indications of substances chemically related to BTMPS (i.e. containing a tetramethylpiperidine moiety) were noted suggesting they resulted from a similar manufacturing process to that used for making illicit fentanyl. Tetramethyl-4-piperidinol was first detected in August 2024 and has appeared in more than 20 drug materials. Tetramethyl-4-AP and tetramethylnorfentanyl were first detected in April 2025 and since have appeared in more than ten drug materials, often together. To date, a substance suspected of being tetramethylfentanyl has been identified in only small amounts alongside these other related substances; however, confirmation of this substance is pending acquisition of a standard reference material.

Discussion: Toxicity and adverse effects in humans associated with BTMPS and its related substances remain unclear and warrant further research, although exposure to it has been linked to adverse cardiac effects in animal models, and drug users exposed to BTMPS report various unpleasant somatic effects. It has no established psychoactive effects. Due to its chemical properties, the detection of BTMPS in blood is more challenging than traditional drugs; however, BTMPS and BTMPS-related byproducts have been detected by routine comprehensive and non-targeted toxicology screening approaches using high resolution mass spectrometry. The activity and potency of tetramethylfentanyl are currently under evaluation. Laboratories should consider monitoring for these substances as surveillance efforts continue and intelligence gathering remains underway.

Conflict of Interest

I receive a salary from NMS Labs, a commercial forensic toxicology reference laboratory.

P-110

Detection of Ortho-Methylfentanyl in Postmortem Cases in Michigan: A Surveillance Report of an Emerging Synthetic Opioid

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Abstract

Introduction: The rapid and continual evolution of synthetic opioids, along with the increasing prevalence of fentanyl analogs, presents significant analytical testing challenges and heightens public health concerns. An emergent substance, ortho-methylfentanyl (OMF), is a novel fentanyl analog characterized by a methyl substitution of its aromatic ring. Its structural resemblance to other fentanyl derivatives and its absence from routine toxicology panels increases the risk of OMF going undetected in forensic investigations. The Swift Toxicology of Overdose Related Mortalities (STORM) program, an opioid surveillance program conducted at Western Michigan University Homer Stryker M.D. School of Medicine, began testing for OMF, on April 1, 2025, resulting in five confirmed OMF cases in Michigan. This study investigates the identification of OMF in postmortem samples with a comparison to other opioids and non-opioids surveyed during the same period.

Objectives: This study documents the presence of OMF in postmortem specimens collected from decedents in three non-contiguous counties in Michigan. This work also raises awareness of the potential for OMF exposure and its apparent spread across Michigan and highlights the need for comprehensive detection strategies and enhanced toxicological surveillance.

Methods/Materials: This study analyzed postmortem blood samples collected by medical examiner offices that were participants of the STORM program. The STORM catchment area was more than half of the 83 counties in Michigan. The STORM test profile included a total of 68 drug/drug metabolites. Samples were analyzed using a solid phase extraction (SPE) technique and subsequent liquid chromatography–tandem mass spectrometry (LC-MS/MS) analysis. In brief, deuterated internal standards were added to samples and quality control material, then extracted, and SPE extracts were dried, reconstituted and analyzed via LC-MS/MS. Chromatographic separation was accomplished using a bi-phenyl column maintained at 40°C and electrospray ionization operated in positive ion mode. Two ion transitions were monitored for each targeted analyte. Chromatographic peak retention time, transition mass information, and peak area ratios were used to identify and qualitatively report results.

Results: From April 1 to May 29, 2025, 665 postmortem samples were analyzed, OMF was identified in five cases (0.84%) of the Michigan submitted casework. The five positive OMF cases originated from three counties, one located in the western part of the Upper Peninsula, another situated in the Lower Peninsula, and the other in the southeastern corner of the Lower Peninsula. All five cases exhibited positive results for fentanyl, norfentanyl, and 4-ANPP; three of these tested positive for para-fluorofentanyl.

Discussion: The presence of OMF was detected in less than 1% of cases; however, its distribution across various counties indicates a concern for regional prevalence. The co-occurrence of OMF with fentanyl and para-fluorofentanyl may suggest shifting trends within the illicit drug market, potentially highlighting intentional mixing or contamination during synthesis. The inclusion of OMF in toxicology panels addresses the urgent need for enhancements in surveillance

systems and the need for expansion of mass spectrometry libraries; collectively, these measures may lessen the underreporting of novel opioids involved in opioid related fatalities.

The identification of OMF in postmortem examinations across multiple counties in Michigan likely signifies the infiltration of a synthetic opioid into the illicit drug supply. Its capacity to elude conventional toxicology screenings reveals a substantial gap between the advent of new analogs and our current detection capabilities. The simultaneous presence of various fentanyl analogs in the same case may illustrate a transformative pattern in drug manufacturing and distribution. As the synthetic opioid landscape continues to evolve, these findings accentuate the pressing necessity for toxicological surveillance to align with the emerging substances it endeavors to identify.

P-111

Four-Year Comparison of Delta-8- and Delta-9-Tetrahydrocannabinol in DUI and Homicide Cases

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Abstract

Introduction: Cannabinoids continue to be among the most widely used recreational drugs in the United States. While delta-9-tetrahydrocannabinol (Δ9-THC) is approved for recreational use in 24 states, Δ8-THC—a structural analog of Δ9-THC—is legal in 26 states, including several where Δ9-THC remains prohibited, such as Alabama. Consequently, delta-8-tetrahydrocannabinol (Δ8-THC) has emerged as a popular legal substitute for Δ9-THC in these jurisdictions

Objectives: This study aims to evaluate the prevalence of Δ8-THC and Δ9-THC in casework analyzed by the Alabama Department of Forensic Sciences over a four-year period.

Methods: Antemortem and postmortem homicide cases submitted between 2021 and 2024 were retrospectively reviewed. Initial screening for cannabinoids in blood and oral fluid was conducted using enzyme immunoassay (EIA) on either a Tecan Freedom Evo75 platform with Immunalysis reagents or a Randox Evidence Analyzer. Confirmatory analysis for Δ9-THC was carried out quantitatively, while Δ8-THC was assessed qualitatively. Both confirmations employed liquid-liquid extraction followed by liquid chromatography-tandem mass spectrometry (LC-MS/MS). The limits of detection (LOD) were 1.0 ng/mL in blood and 0.5 ng/mL in oral fluid for Δ9-THC and 1.0 ng/mL for Δ8-THC in both blood and oral fluid.

Results: In 2024, data from the Alabama Department of Forensic Sciences Toxicology Department revealed that Δ9-THC was the second most frequently detected drug in driving under the influence (DUI) and traffic fatality cases. Δ8-THC ranked seventh in DUI cases and fourth in traffic fatalities. Over the four-year period, 6,475 antemortem blood samples, 2,087 homicide postmortem blood samples, and 1,493 oral fluid specimens from DUI suspects were analyzed. Δ9-THC was detected in 1,926 (30%) antemortem blood cases, 912 (44%) postmortem homicide cases, and 879 (59%) oral fluid DUI cases. Δ8-THC was found in 333 (5%) antemortem cases, 45 (2%) homicide postmortem cases, and 140 (9%) oral fluid cases. Of the 378 total blood cases positive for Δ8-THC, 298 (79%) also contained Δ9-THC, indicating notable co-use. Specifically, co-occurrence of Δ8-THC and Δ9-THC was observed in 259 (4%) antemortem, 39 (2%) homicide postmortem cases, and 116 (8%) of oral fluid DUI cases. Despite Δ9-THC's higher prevalence, Δ8-THC was identified in approximately 4% of blood-positive cannabinoid antemortem and postmortem homicide cases and 9% of oral fluid samples.

Cases Analyzed Between 2021 - 2024			
	All Cases Δ9-THC Positive	**All Cases Δ8-THC Positive**	**Cases Positive for Both Δ8-THC & Δ9-THC**
Blood – Antemortem & Homicide Postmortem	2,838 (33%)	378 (4%)	298 (3%)
Antemortem - Blood	1,926 (30%)	333 (5%)	259 (4%)
Homicide Postmortem - Blood	912 (44%)	45 (2%)	39 (2%)
Oral Fluid	879 (59%)	140 (9%)	116 (8%)

Discussion: The data demonstrates that Δ9-THC continues to be the predominant cannabinoid detected in forensic casework in Alabama; however, the presence of Δ8-THC in both antemortem and homicide postmortem specimens has increased, highlighting its emerging role as a legal alternative in jurisdictions where Δ9- THC remains illegal. The frequent co-detection of Δ8-THC and Δ9-THC suggests overlapping use patterns and potential challenges in distinguishing between legal and illicit cannabinoid exposure. These findings underscore the need for continued surveillance and analytical differentiation of cannabinoid isomers in forensic toxicology. Notably, Alabama has enacted some of the most stringent regulations on cannabinoid products, most recently with the passage of House Bill 445 in 2025.

P-112

Automated Extraction and Chromatographic Separation of Δ 8, 9, 10 THC Isomers from Whole Blood

Kyle Dukes[1], Esraa AboJasser[1], Sohel Rana[1], Lee Williams[2]

[1]Biotage, Charlotte, NC, USA. [2]Biotage, Cardiff, United Kingdom

Abstract

Introduction: Analysis of tetrahydrocannabinol (THC) and its isomers is crucial for understanding their pharmacological effects and legal implications of cannabis use, especially in clinical testing, forensic toxicology, and sport antidoping. THC, the primary psychoactive component of cannabis, exists in several isomeric forms, including Δ9, Δ8, and Δ10-THC, each with distinct pharmacokinetic and pharmacodynamic properties. Accurate identification and quantification of these isomers are essential for evaluating impairment, exposure, or intoxication. However, their analysis presents significant challenges due to their chemical similarities, which can result in co-elution during chromatographic separation and difficulty in distinguishing between isomers using traditional analytical methods.

Objectives: This work will demonstrate extraction strategies for cannabigerol, Δ8, Δ9, and Δ10 THC, as well as the common metabolites 11-hydroxytetrahydrocannabinol (11-OH-THC) and 11-nor-9-carboxy-tetrahydrocannabinol (THC-COOH) from whole blood. In addition, two chromatographic methods of separating the isomers will be presented.

Methods: Δ9, Δ8, and Δ10-THC and the metabolites 11-hydroxytetrahydrocannabinol (11-OH-THC) and 11-nor-9-carboxy-tetrahydrocannabinol (THC-COOH) were spiked in whole blood samples concentrations ranging from 0.15-100ng/mL and extracted using ISOLUTE® SLE+ supported liquid extraction plates. Extraction performance was investigated by comparing recovery and matrix effects using MTBE, EtOAc, EtOAc followed by Hexane, and Hexane followed by EtOAc as an elution solvent. Optimized extraction protocol was automated on the Biotage® Extrahera™ automated sampled preparation workstation. Chromatographic separation was investigated by comparing two different analytical column chemistries, biphenyl and fluorophenyl. Protocols were transferred to the Extrahera™ Classic automated sample preparation workstation and evaluated for linearity and precision. UHPLC-MS/MS analysis was performed using a Shimadzu Nexera X2 UHPLC coupled to a Sciex 5500 triple quad mass spectrometer. In addition, column chemistry and oven temperature were investigated to achieve optimal separation of the isomers.

Results: Extraction performance was investigated by comparing recovery and matrix effects using the follow elution solvents: MTBE (750µLx2), EtOAc (750µLx2), 750 µL EtOAc followed by 750 µLHexane, and 750 uL Hexane followed by 750 µL EtOAc. While these four elution protocols yielded similar matrix effects, EtOAc achieved the highest recoveries of analytes, 70-90% with minimal matrix effects (0.9-1.2). The optimized extraction procedure using ISOLUTE® SLE effectively removed more than 99% of the phospholipids present compared to the protein precipitation method. Final automated method performance and calibration curves demonstrated excellent linearity with coefficients of determination, r2 > 0.99 and excellent reproducibility with RSDs below 10% for all analytes.

Discussion: THC and its metabolites are effectively extracted by supported liquid extraction due to favorable partitioning into the organic extraction solvent layer from the aqueous whole blood matrix. The SLE workflow is comparatively simple compared to a traditional SPE approach which requires additional conditioning, equilibration, and wash steps. Baseline separation of THC isomers can be challenging. Investigation of column chemistry showed that fluorophenyl chemistry achieved better resolution of isomers than biphenyl. Moreover, investigation of column oven temperature demonstrated that a lower oven temperature, 20°C, is more effective in separating THC isomers and its metabolites.

Conflict of Interest

Salary

P-113

Sample Cleanup Approach for the Analysis of A Multi-Class Drugs of Abuse Panel in Breast Milk

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Abstract

Introduction: Breastfeeding offers significant health benefits for both infants and mothers, promoting optimal nutrition, immune protection, and developmental outcomes. However, maternal use of illicit drugs or misuse of prescription medications during lactation can result in the transfer of potentially harmful substances into breast milk, posing serious risks to the nursing infant. Consequently, the ability to accurately detect and quantify drugs of abuse in breast milk is critical for clinical toxicology, forensic investigations, and public health monitoring.

Objectives: This study demonstrates a robust and sensitive analytical workflow for the extraction and detection of commonly encountered drugs of abuse in human breast milk with high specificity, low detection limits, and reliable quantification across the range of analytes.

Methods: A panel of 55 analytes with a diverse chemical backbone and a wide range of LogP (-0.6-7.05) and pKa (0.13-10.47) was spiked into breast milk aliquots. Sample extraction was investigated by comparing protein precipitation, cSPE for QuEChERS, and other SPE techniques. The optimized protocol was transferred to the Extrahera® automated sample preparation workstation. Subsequent analysis was performed using a Shimadzu Nexera X2 UHPLC system coupled to a SCIEX 5500 QTrap MS system. Extraction performance was evaluated by evaluating extract recovery, matrix effect, and reproducibility.

Results: We observed consistent extraction performance using the cSPE for QuEChERS workflow, with the majority of investigated analytes achieving 70%-90% recovery with excellent reproducibility (RSD=6-15%). The cSPE QuEChERS extraction effectively removed over 99% of the phospholipids that co-extracted during protein precipitation, while maintaining excellent extraction recovery for a diverse range of analytes. More importantly, the cSPE QuEChERS extraction is simpler and faster than traditional SPE. Completing a 96-well plate, the cSPE QuEChERS extraction required just two steps and 45 min, compared to the five steps and 75 minutes needed for the SPE extraction.

Discussion: The overall cSPE QuEChERS sample extraction efficiency was influenced by the physical interaction between biomatrix and the extraction solvent, acetonitrile. Increasing the sample/solvent ratio, dispensation force, and pipette mixing speed significantly improved the extraction of all analytes, resulting in less variability. Final method performance and calibration curves demonstrated excellent linearity and coefficients of determination, $r^2 > 0.99$ for all analytes.

Conflict of Interest

Salary

P-114

LC-MS/MS Analysis of Benzodiazepines in Urine with Room Temperature Hydrolysis and One-Step Clean Up Using Beta -Gone

Andre Sukta[1], Elias Villalobos[2], Crystalyn Consolacion[3], Stephanie Marin[3]

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Abstract

Introduction: Benzodiazepines are a class of depressants that are used for sedation, to relieve anxiety, and reduce muscle spasms and seizures. This class of compounds is considered to be a controlled substance since they have the potential to be habit-forming and abused. Toxicological testing for benzodiazepines can be helpful in drug monitoring, drug-facilitated crime, impairment investigations, and cause of death determinations.

Objectives: Urine is one of the most commonly used specimens for drug testing. Benzodiazepines are extensively metabolized through methylation and hydroxylation, and they are heavily conjugated before excretion in urine. The goal of this presentation is to demonstrate a fast, low-volume, and reproducible method for analyzing benzodiazepines in urine using room-temperature hydrolysis and an LC-MS/MS method.

Methods: Eight calibrators, ranging from 20 to 2500 ng/mL, and three QC samples at 50, 125, and 800 were prepared in drug-free urine (7-aminonitrazepam, 7-aminoclonazepam, 7-aminoflunitrazepam, alpha-hydroxy midazolam, alpha-hydroxy triazolam, alpha-hydroxy alprazolam, oxazepam, lorazepam, 2-hydroxy ethylflurazepam, temazepam, nordiazepam). Internal standard (IS) concentrations were 100 ng/mL for all analytes (d4-7-aminoclonazepam, d5-alpha-hydroxy alprazolam, d5-oxazepam, d4-triazolam, d5-temazepam, d5-nordiazepam). A total of 100 µL of urine was aliquoted, and 50 µL of IS was added along with 200 µL of Kura B-One enzyme. The samples were incubated at room temperature for 30 minutes. Before transfer to an autosampler vial for analysis on the LC-MS/MS, the samples were filtered through the Beta-Gone 1 mL cartridge.

The analysis was performed on an Agilent 1200 series liquid chromatography system coupled with a Sciex 5500 Triple Quad mass spectrometer. A Kinetex Phenyl-Hexyl column, measuring 50 x 4.6 mm and 2.6 µm (Phenomenex), was utilized for chromatographic resolution of the analytes. Mobile phase A consists of 10 mM Ammonium Formate with 0.05% Formic Acid, while mobile phase B is methanol containing 0.05% Formic Acid. The flow rate was set at 0.6 mL/min, with an injection volume of 3 µL, and the column heater was maintained at 40°C.

Table 1: Gradient

Time (min)	%B
0.0	10
4.5	100
5.5	100
6.1	10
7.5	10

The mass spectrometer ion source was a turbo spray with an ESI in positive polarity mode. The source parameters are as follows: source temperature 650°C, Gas 1 60, Gas 2 50, curtain gas 40, ion spray voltage 2500.

Table 2: MRM

	Q1	Q3	Dwell	DP	CE	CXP
7-aminonitrazepam	252.10	121.00	15.00	40.00	23.00	35.00
7-aminoclonazepam	286.10	250.00	15.00	80.00	19.00	13.00
7-aminoflunitrazepam	284.10	135.10	15.00	51.00	23.00	39.00
alpha-OH Midazolam	342.10	203.10	15.00	60.00	26.00	10.00
alpha-OH Triazolam	359.10	331.10	15.00	90.00	27.00	12.00
alpha-OH Alprazolam	325.10	297.10	15.00	60.00	30.00	15.00
Oxazepam	287.10	241.10	15.00	76.00	31.00	18.00
Lorazepam	321.10	275.10	15.00	80.00	30.00	10.00
2-OH Ethylflurazepam	333.10	211.20	15.00	51.00	25.00	53.00
Temazepam	301.10	255.10	15.00	70.00	15.00	8.00
Nordiazepam	271.10	140.10	15.00	100.00	30.00	15.00

Results: The matrix effect ranged from 22% to 49%, and recovery varied from 94% to 109%, with %CVs of ±15% for analytes with a corresponding isotopically labeled internal standard and within ±20% for analytes without a matching internal standard (n = 12). Hydrolysis efficiency was 100% for an oxazepam glucuronide control.

Discussion: The rapid 30-minute room temperature hydrolysis using Kura B-One® and a straightforward one-step extraction with Beta-Gone yielded very clean samples with high recovery, low matrix effect, and excellent hydrolysis efficiency. The quick 5-minute LC separation and LC-MS/MS analysis utilizing a SCIEX® 5500 MS/MS system delivered accurate and reliable quantification of benzodiazepines in urine.

Conflict of Interest

Authors are paid salary by their respective companies and products that their company sells is represented in the presentation.

P-115

Enhancing Forensic Toxicology Through Post-Mortem Applications: Evaluating the Viability of Skin Cells as a Drug Testing Matrix

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Abstract

Introduction: The Centers for Disease Control reported that 70.3 million individuals abused drugs, resulting in 89,740 overdose-related deaths in 2024. Implications of drug abuse—relapse, recurring use, emotional and physical alterations, lack of motivation, unpredictability, lack of self-care, and loss of control—have influenced drug testing practices through real-world applications, including workplace safety, treatment monitoring, relapse prevention through detection, court-ordered programs, and criminal investigations. Specifically, law enforcement organizations, such as the Federal Bureau of Investigation, reveal drug abuse patterns through smuggler routes and networks, polydrug trade, adulterated substances, demographic user variation, cross-addiction, and sale platforms amongst drug trafficking, manufacturing, distribution, and composition. Frequently abused drugs within this application include amphetamines, cannabinoids, cocaine, opiates, phencyclidine, oxycodone, methadone, buprenorphine, fentanyl, psychedelics, and morphine. While blood and urine are the gold standards, the current drug testing matrices pose limitations of time, invasiveness, required materials/collection professionals, cost requirements, and accuracy. The limitations prompt reconsideration of the optimal matrix and exploration of novel alternatives.

Objectives: This research aims to examine the viability of utilizing skin cells as an effective method for extracting, detecting, and quantifying illicit substances. Additionally, it tests the null hypothesis by way of a p-value test and performs a six-way ANOVA test between each matrix, focusing on the factors of time, cost, and efficiency.

Methods: Ten samples were collected from five overdose decedents at the Westmoreland County Forensics Laboratory: four skin cell samples, two oral fluid samples, one vitreous humor sample, one blood sample, one hair sample, and one urine sample. The skin cell samples were collected via cotton swabs from the hairline, neck, armpits, and behind the knees. All samples were individually stored in their respective 10 mL plastic tubes at 4°C until extraction was performed, except for the hair sample, which was stored in a manilla envelope at 25°C. Apart from the skin cells, each matrix underwent different previously established solid phase extraction methods. The skin cell extraction method combines DNA extraction methods for buccal samples from cotton swabs and toxicology methods for solid phase extraction from tissue samples.

The Agilent 1200 Series LC Stack and 6460 Triple Quad Mass Spectrometer were used in conjunction with Multiple Reaction Monitoring (MRMs) to identify the presence of seven target drugs: fentanyl, morphine, 6-monoacetylmorphine, oxycodone, methamphetamine, alprazolam, and benzoylecgonine. Quantification was completed using a calibration curve derived from peak height ratios of the nondeuterated and deuterated target drugs in nine standards, with

concentrations from 1500 ng/mL to 5.86 ng/mL. All chromatograms were analyzed using MassHunter Workstation Qualitative Analysis software.

The results' validity was determined by cross-referencing the Westmoreland County Forensics Laboratory toxicology reports. A 6-way ANOVA test will be conducted to determine which matrix is most suitable based on cost efficiency, testing duration, and effectiveness. A 95% confidence interval and *p*-value testing will be administered to determine statistical significance and whether the null hypothesis, skin cells are not a viable matrix for drug testing, would be rejected or fail to be rejected.

Results: Currently, results from preliminary testing produced chromatographs revealing skin cells as a viable method for drug testing, as they yielded prominent peaks viable for identification and quantification for methamphetamine, morphine, oxycodone, benzoylecgonine, and alprazolam. The skin cell extraction method utilizes procedures that are congruent with DNA extraction by employing a homogenous mixture, proteinase K, a heating period, and lysis buffer to produce accurate concentrations and viable chromatograms for the remaining target drugs. Chromatograms produced from the established matrices prove their methods yield adequate results.

Discussion: Preliminary results suggest skin cells are a viable matrix for a noninvasive and cost-effective method of drug testing. While this research only utilizes postmortem individuals as its subjects, introducing a potential gap in the field, it is hypothesized that findings will be reciprocal to living subjects. However, further research, where this study is repeated on living donors, is required to verify this hypothesis. Overall, the impact of this research permits the designation of the most adequate matrix while introducing an additive matrix that would augment existing drug testing options.

P-116

Development of a Quantitative Method for Opioids in Blood Using LC-MS/MS and Solid Phase Extraction

Alexus Ramirez-Wiggins[1,2], Jessica Ayala[2], Dayong Lee[2], Dani Mata[2]

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Abstract

Introduction: Currently, HFSC uses two solid phase extraction (SPE) techniques and liquid chromatography tandem mass spectrometry (LC-MS/MS) methods for detecting a range of opioids in blood and urine including morphine, oxymorphone, hydromorphone, codeine, o-desmethyltramadol, 6-acetylmorphine, oxycodone, hydrocodone, tramadol, norbuprenorphine, buprenorphine, norfentanyl, fentanyl, 2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine (EDDP), and methadone (Houston Forensic Science Center, 2024). Having two opioid detection methods requires the need for more reagents and solvents, gases, equipment and materials, extraction columns, standards/ solutions, and uses more of our analyst's time for extraction, processing, and review. Another caveat of having two separate methods is the potential double sampling of a case.

Objectives: The purpose of this study was to improve the current case workflow and combine the two existing opioid methods for detecting 15 different opioids in blood and urine.

Methods: The extraction method utilized a solid phase extraction using QCT Clean Screen columns and protein precipitation. One milliliter of blood or urine was fortified with analytes of interest, and a protein precipitation was completed with 2 mL of cold acetonitrile. Samples were then centrifuged, the supernatant was diluted with 3 mL of 100 mM sodium phosphate buffer, and the samples were then added to the SPE columns. Multiple solvents were used to wash the samples, and drugs were eluted with methylene chloride: isopropanol: ammonium hydroxide (78:20:2). Eluates were evaporated under nitrogen and reconstituted with 150 µL of 95:5 (A: B), mobile phase A water with 5mm ammonium formate and 0.05% formic acid and mobile phase B acetonitrile with 0.1% formic acid The LC method begins with 95% A , at 2 minutes the aqueous mobile phase decreases to 80%, then at 9 minutes it is as 50 % A, and lastly it will go to 2% A at 11 minutes and hold till 13.50 minutes.

Results: Development tests included extraction and instrument method optimization. Validation experiments will follow the ASB 036 Standard for quantitative analysis. The limit of quantitation will be administratively set at the cut-off concentrations listed in ANSI/ASB Standard 120 for blood and ANSI/ASB 121 for urine.

Discussion: One limitation of the new method is the need to have washes in between each injection due to potential carryover, which could not be removed with changes to the flow rate or instrument washes. On the other hand, the current methods use two different LC columns which are a C18, 2.1 x 50 mm and a Biphenyl, 2.1 x 100 mm while the new method needs one column C18 2.1 X 100 mm. The current methods use three different mobile phases between the two methods, but the new method requires just two. The added benefit of only one analyst needed to analyze and review a batch for all commonly detected opioids in a case sample helps the laboratory reduce turnaround time.

EXHIBITORS

COMPANY/ORGANIZATION	BOOTH #
9-Delta Analytical LLC & Responsibility.org	404
Abbott	302
Absolute Standards, Inc.	805
Agilent Technologies	300
American Solutions for Business	106
ANSI National Accreditation Board	701
ARK Diagnostics, Inc.	307
AXIOM DIAGNOSTICS, Inc.	102
Biotage, LLC	705
Bruker Scientific LLC	204
Campbell Science	203
Cayman Chemical Company	405
Collaborative Testing Services	107
Diamond Diagnostics	607
DPX Technologies, Inc.	306
DrugImpairment.com	205
EquipNet, Inc.	605
Forensic Advantage Systems	703
Golden West	400
IMCS	601
Indigo BioAutomation	402
Innovative Lab Services	803
INTEGRA Biosciences	700
ionBench Corporation	206
JEOL USA, Inc.	303
Kura Biotech	503
LGC Standards	802

COMPANY/ORGANIZATION	BOOTH #
Lin-Zhi International, Inc.	403
LNI Swissgas	604
Medix Biochemica	207
Mercedes Scientific	804
MilliporeSigma	807
Neogen	702
NMS Labs/CFSRE	200 & 202
Peak Scientific	108
Peeke Scientific	406
Phenomenex Inc.	600
Phytronix Technologies Inc.	407
Randox Toxicology	502
Restek	806
SCAT Americas, Inc.	208
SCIEX	500
Shimadzu Scientific Instruments Inc.	304
Siemens Healthineers	505
SOFT-TIAFT 2026 Chicago	603
Tecan	506
Thermo Fisher Scientific	606
UCT	104
UTAK Laboratories, Inc.	401
Validity Diagnostics	704
Versaterm	706
VICI DBS	800
Waters Corporation	100

TIER I TIER II TIER III TIER IV TIER V FUN RUN/WALK

OREGON CONVENTION CENTER
EXHIBIT HALL D | LEVEL 1
EXHIBITORS

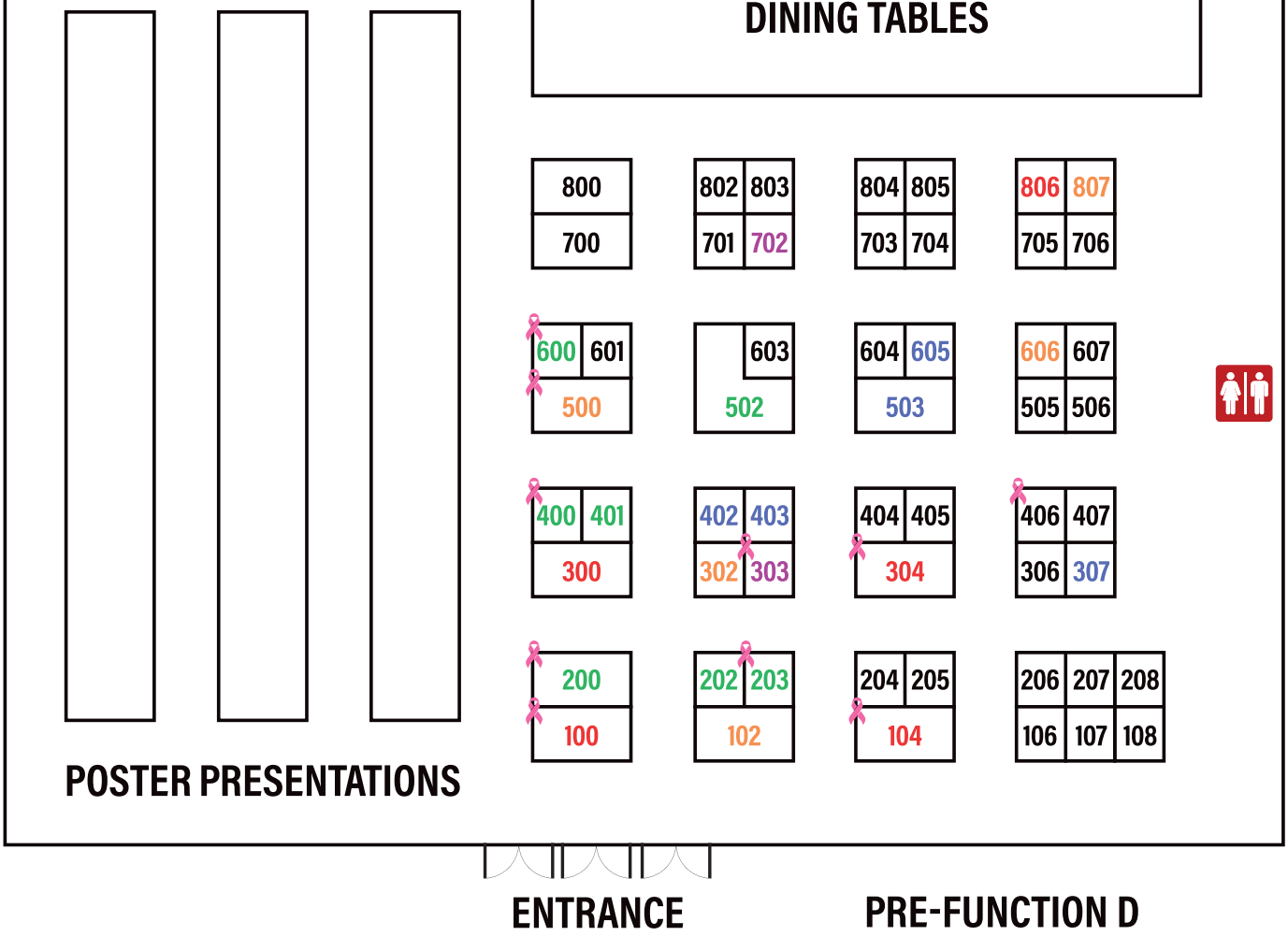

EXHIBIT HALL HOURS

Tuesday, October 28 | 6:30-9:30PM

Wednesday, October 29 | 9:30AM-4:00PM

Thursday, October 30 | 9:30AM-3:30PM

SPONSORSHIP

TIER I SPONSOR $15,500
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TIER III SPONSOR $3,500
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FUN RUN/WALK SPONSORSHIP

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EXHIBITORS

ABBOTT

Booth 302

Tier II Sponsor

https://www.toxicology.abbott/us/en/solutions/clinical-toxicology-solutions.html

There is nothing more personal than our health. With a comprehensive range of toxicology reagents, Abbott equips you with relevant tools to support the health and wellbeing of your patients.

Abbott develops and manufactures immunoassay reagents for the detection of drugs and alcohol in a variety of biological matrices such as oral fluid, urine, and blood for use in clinical, criminal justice, workplace, and drug treatment settings.

Our reagents are provided in three ready-to-use formats (our exclusive HEIA™, high-sensitivity SEFRIA™, and classic ELISA) and are available in multiple kit sizes including convenient 60 mL wedges for use on high volume analyzers. With the largest panel of ELISA and HEIA™ oral fluid reagents, Abbott also offers the best-in-class Quantisal™ and Quantisal™ II Oral Fluid collection devices, with easy-to-read volume adequacy indicators.

Abbott also provides an extensive line of rapid drug testing solutions, including instant-read urine cups, cassettes, and surface wipes.

Our complete line of assays, calibrators, and controls provides cost-effective and efficient drug testing solutions for today's laboratories.

ABSOLUTE STANDARDS, INC.

Booth 805

60 Trigo Drive
Middletown, CT 06457

Stephen Arpie, Director

stephenarpie@absolutestandards.com

www.absolutetstandards.com

Absolute Standards, Inc. is a manufacturer of Certified Reference Materials and Proficiency Tests. Our ISO Accreditations in 9001, 17025, 17034, and 17043 are your assurance of true value and quality. With 35 years of experience, Absolute Standards has the technical expertise, customer service, and commitment to quality it takes to ensure your laboratory has what it needs to stay calibrated and compliant with ever-changing environmental and legislative demands. The range of calibration standards includes organic and inorganic materials for use in environmental, forensic, drug, energy, cannabis, and other industries. We offer a complete line of single components solutions and specialize in multicomponent mixtures to maximize every run. Custom solutions for SAMHSA, CAP, and other drug testing are readily available to fit specific analysis requirements. If you need the standard, we have the solution.

EXHIBITORS

AGILENT TECHNOLOGIES

Booth 300

Tier I Sponsor

www.agilent.com

Agilent Technologies leads the industry with robust, reliable instruments that provide the ability to analyze, confirm and quantify substances of interest. Our workflow solutions enable you to maintain stringent practices from sample preparation, through analysis, to final report. When combined with our informatics architecture, large quantities of data can be managed while preserving the integrity and security of the results. Agilent offers a complete line of GC, LC, MS and Spectroscopy instruments and technologies, as well as the related consumables, support and services. For more information, visit www.agilent.com.

AMERICAN SOLUTIONS FOR BUSINESS

Booth 106

901 Penamint Court
Chanhassen, MN 55317

952-470-1020

Kathy Petrick

KathyPetrick@msn.com

www.americanbus.com

American Solutions for Business is a leading distributor of custom business documents and labels, specializing in Federal and Non-Federal Chain of Custody forms and labels for forensic toxicology, pain management, and other laboratory applications.

Our comprehensive lab solutions include:

- Tamper-evident labels, accession labels, and freezer-grade adhesives
- Traditional multi-part Chain of Custody forms
- A universal forensic all-in-one label
- Technology-enabled solutions such as OCR-scannable documents, QR codes, and high-performance adhesives designed specifically for lab environments

With over 25 years of industry experience, Program Manager Kathy Petrick delivers unmatched knowledge and customer support in the forensic toxicology and drug testing space. We offer:

- Exceptional customer service
- Competitive pricing
- Deep understanding of regulatory and lab needs

In addition to lab products, American provides a full range of branded promotional items, including:

- Bags, drinkware, backpacks, lunch totes
- Lab coats, custom apparel, socks
- Lapel pins, writing instruments, and more

We are proud to be a supplier of promotional products and conference apparel for the SOFT Conference for several years running.

EXHIBITORS

AXIOM

Booth 102

Tier II Sponsor

4309 West Tyson Ave
Tampa, FL 33611

Jesse Carter

info@axiomdiagnostics.com
support@axiomdiagnostics.com

www.agilent.com

Axiom Diagnostics announces new TEST TRUE synthetic matrices, HemaSyn, SeraSyn, OralSyn, MecoSyn, and UrinSyn, engineered to mirror blood, serum, saliva, meconium, and urine. They include the indigenous chemical compounds and physical characteristics your methods expect, without unwanted components from pooled human/animal biologics—ideal for QA and calibration substrates. These synthetic duplicates deliver reliable, consistent results at lower cost. (SVT tests for saliva coming soon.)

For 25+ years, Axiom has led Sample Validity Testing. We've expanded our SVT portfolio with new Linearity Standards kits for creatinine, pH, specific gravity, nitrite, chromate, and oxidants. The Test True™ line of assays, calibrators, and controls remains a proven, affordable choice for detecting adulterants in DAU urine testing, including assays for nitrite, pH, SG, chromate, creatinine, aldehydes, oxidants, and synthetic urine.

Our Synthetic Urine Assay identifies ~95% of synthetic urines on the market (MRO Alert, Oct. 2015). Our Oxidant Assay detects nitrite, chromate, bleach, iodic acid, iodate, peroxide, and more, and has outperformed competitors (MRO Alert, Oct. 2013). TrueTrol multi-level controls and SG controls meet SAMHSA QA requirements. Custom controls and packaging available.

Visit our booth to see what's new and don't miss the annual wine giveaway!

BRUKER SCIENTIFIC LLC

Booth 204

With a focus on the food, environmental, forensic toxicology, clinical research, polymers and industrial markets, the new Bruker Applied Mass Spectrometry division is focused on developing workflows and solutions that meet the unique needs of these users. Analytical areas such as characterization, safety, authenticity, adulteration, QC of starting and finished goods are amongst the applications covered and used across industrial, government and academic institutes. The Bruker Applied Mass Spectrometry division is dedicated to helping provide rugged and reliable workflows and complete solutions that simplify and make users more efficient and productive.

EXHIBITORS

CAYMAN CHEMICAL COMPANY

Booth 405

1180 East Ellsworth Road
Ann Arbor, MI 48108

Rikki Walter

sales@caymanchem.com

https://www.caymanchem.com/forensics/

Cayman specializes in providing high-purity analytical reference standards and resources that support forensic toxicologists worldwide. We offer an industry-leading collection of forensic and toxicology reference standards, including traditional drugs, novel psychoactive substances (NPS), and their metabolites. Cayman products are available as fit-for-purpose analytical standards and ISO 17034 Certified Reference Materials.

Visit our booth and Explore:

- Comprehensive Screening Solutions – Collections of standards enabling advanced laboratory detection, including 96-well tube rack formats and multi-component mixtures
- Drug Metabolites & Metabolism Studies – Expertise in developing standards for probable drug metabolites, including emerging semi-synthetic cannabinoids and brorphine-based synthetic opioids
- NPS Unknown Identification – Free, one-on-one support in the identification of MS fragmentation patterns from our experienced forensic chemists
- Custom Synthesis – Tailored solutions to meet the evolving needs of forensic toxicologists

COLLABORATIVE TESTING SERVICES

Booth 107

Jenna McConnell

jenna.mcconnell@cts-interlab.com

https://cts-forensics.com/index-forensics-testing.php

Founded in 1971, Collaborative Testing Services (CTS) is an ISO 17043-accredited proficiency testing provider that serves the evolving quality assurance needs of several industries with innovative interlaboratory proficiency testing. With worldwide clients in both the public and private sectors, we aim to create and administer sustainable and meaningful testing schemes for drug analysis, forensic biology and DNA, firearms and toolmarks, latent prints and impressions, questioned documents, trace evidence, toxicology, crime scene analysis, digital and multimedia evidence, and forensic anthropology.

In addition to our commitment to quality proficiency testing, we are dedicated to leadership in our industry. As a 100% employee-owned small business, we foster a growth-oriented culture where our staff are empowered by their colleagues and motivated by their direct contributions to our success.

EXHIBITORS

DRUGIMPAIRMENT.COM

Booth 205

Matthew Myers, MS, MPA

Info@DrugImpairment.com

https://DrugImpairment.com

DrugImpairment.com connects toxicologists and law enforcement professionals with industry leading instructors and advanced education on drug impairment in a platform that's mindful of limited budgets and scheduling challenges. Courses are led by many of the most prominent names in the industry and delivered through an e-learning platform that allows users to learn at their own pace and convenience. Everything in our continually growing catalog is included with membership, so there are no per-course fees. Continuing education certificates are granted upon completion and widely accepted for CEU credits from different credentialing bodies, including ABFT. Group accounts are available, allowing local agencies or state programs to get training for their whole team and self-administer learner accounts and progress. DrugImpairment.com also offers supporting resources that are purpose-built for forensic practitioners to get the information they need about drugs from a credible source without having to scour the web. Learn more at https://DrugImpairment.com

EQUIPNET, INC.

Booth 605

Tier IV Sponsor

https://www.equipnet.com/

EquipNet is the world's leading provider of surplus asset management solutions, specializing in helping forensic toxicology and analytical laboratories unlock value from idle or underutilized instrumentation. We support government labs, private labs, academic institutions, and healthcare systems by offering customized programs for selling, redeploying, or sourcing certified pre-owned LC/MS/MS systems, GC, HPLC, and other analytical equipment. Whether your lab is consolidating, upgrading, or relocating, EquipNet provides global exposure, dedicated project management, and compliance-driven logistics to ensure seamless transitions. Our solutions not only generate capital but also promote sustainability and operational efficiency. With over 20 years of experience and a global buyer network, EquipNet empowers labs to reinvest in innovation while reducing waste.

EXHIBITORS

GOLDEN WEST

Booth 400

Tier III Sponsor

Matthew Myers, MS, MPA

www.goldenwestbio.com

Golden West Companies (ISO13485:2016 Certified), composed of Golden West Diagnostics, LLC (GWD) and Golden West BioSolutions, LLC (GWS), provide high-quality biological materials and tailored solutions for the in-vitro diagnostic and life science industries. GWD specializes in custom OEM products and human matrices, including the Mass Spect Gold line for ultra-low analyte testing. GWS complements this by supplying a wide range of human donor products, patient samples, and antigens that support research, development, and diagnostic innovation. With almost four decades of expertise, Golden West Companies deliver reliable, cost-effective, and innovative solutions that empower customers with unmatched quality, consistency, and value.

IMCS

Booth 601

110 Centrum Drive
Irmo SC, 29063

inquiries@imcstips.com

Automated high-throughput protein purification with IMCStips® - IMCS

Integrated Micro-Chromatography Systems, Inc. (IMCS) is a privately-held biotech company that designs, manufactures, and distributes biological reagents ranging from recombinant proteins to micro-chromatography consumables. Its two product lines, IMCSzyme® and IMCStips®, serve clients in clinical and forensic toxicology, academic research, government agencies, and life science.

IMCSzyme®, the first genetically modified, industry-disrupting β-glucuronidase, accelerates hydrolysis and improves throughput. The second-generation IMCSzyme® RT enables room temperature hydrolysis of drug metabolites in 15 minutes or less and is uniquely resistant to inhibitors in human urine. To optimize workflows and reduce sample costs, contact inquiries@imcstips.com.

IMCStips, is an SPE-in-a-Tip solution: specialized pipette tips that contain resin used for rapid and reliable automated purification of proteins, antibodies, and enzymes in proteomics, biomarker discovery, and other analytical workflows. IMCS also offers glycosyltransferases to expand the synthesis of glycosphingolipids and sialoglycans for neurodegenerative disease research.

EXHIBITORS

INDIGO BIOAUTOMATION

Booth 402

Tier IV Sponsor

Indigo BioAutomation products and services help independent, hospital and specialty labs and test manufacturers power precision, simplify complexity, and scale performance. ISO 13485:2016 certified since 2015 and trusted in the processing of more than 250 million samples, Indigo's current portfolio includes ASCENT, ARQ and analytics optimized data warehouse capabilities for the tracking, reporting, and analysis of certified batch data. Cloud-based Class 1 (exempt) Medical Devices, ASCENT for GC/LC-MS/MS and ARQ for RT-PCR/qPCR transform moderate- to high-complexity LDT workflows into scalable, consistent, and audit-ready operations with rules-based SOP standardization, lab-wide collaboration, and optimization and alignment across instruments, users, and locations. Designed to evolve with expanding test menus, instrumentation, and compliance expectations, Indigo's systems and software help labs operate with confidence in an increasingly demanding regulatory and scientific environment.

INTEGRA BIOSCIENCES

Booth 700

https://www.integra-biosciences.com

INTEGRA is a leading provider of high-quality laboratory tools and consumables for liquid handling and media preparation. We are committed to fulfilling the needs of laboratory professionals in research, diagnostics, and quality control within the life sciences industry.

INTEGRA's globally recognized products include our EVOLVE manual pipettes, VIAFLO and VOYAGER electronic pipettes, VIAFLO 96/384, MINI 96, ASSIST and ASSIST PLUS, WELLJET reagent dispensers, PIPETBOY serologic pipettors, VACUSAFE and VACUSIP aspiration systems, DOSE IT peristaltic pumps, and lastly our MEDIACLAVE and MEDIAJET media preparation equipment. Visit www.integra-biosciences.com to see our full line of innovative products.

EXHIBITORS

IONBENCH CORPORATION

Booth 206

https://ionbench.com/

ionBench is the expert in standard and tailor-made analytical laboratory furniture.

Our products are manufactured to simplify the daily operation of your LC/GC/ICP/OES/MS systems, offering features and accessories that significantly enhance your work environment.

Create a healthier, more productive environment with an analytical lab designed to your requirements.

- Reduce noise: Integrated noise enclosure, reduce 80% of the noise perception up to -20 dB(A)
- Space Optimization: Maximize your footprint for a seamless workflow.
- Prioritize Safety: Enhanced safety with advanced, built-in features.
- Tailor-made: Designed specifically to meet your unique requirements.

JEOL USA, INC.

Booth 303

Tier V Sponsor

https://www.jeolusa.com

JEOL USA is the North American subsidiary of JEOL Ltd., supporting forensic and toxicology laboratories with mass spectrometry solutions and U.S.-based applications and service. For rapid screening and confirmation, our portfolio includes AccuTOF DART for ambient sampling, the AccuTOF GC-Alpha high-resolution GC–MS, and the JMS-TQ4000GC triple-quadrupole GC–MS/MS for targeted quantitation. These systems help labs address workflows such as seized drug identification (including NPS), postmortem toxicology, and trace/unknown analysis. With more than 75 years of JEOL innovation and a headquarters in Peabody, MA, we collaborate on method development, training, and ongoing support.

EXHIBITORS

KURA BIOTECH

Booth 503

Tier IV Sponsor

www.findenkura.com

At Finden®, we know every test matters, and critical tests require the best enzymes.

Specializing in β-glucuronidase and Aryl-sulfatase, we provide advanced enzymatic solutions for toxicology analysis, making sample preparation faster, simpler, and more consistent. By continuously improving our enzymes, we help laboratories achieve accurate, consistent results while increasing throughput and ensuring better outcomes for patients.

LIN-ZHI INTERNATIONAL, INC.

Booth 403

Tier IV Sponsor

Direct: 408-320-9211

Main: 408-970-8811x1

customerservice@lin-zhi.com

www.lin-zhi.com

Lin-Zhi International, Inc. (LZI) is focused on innovative and quality products for clinical diagnostics. We lead the industry in the development of challenging assays that are liquid and ready to use. These novel assays include our FDA 510(k) cleared 6-Acetylmorphine (6AM), Buprenorphine (BUP), Ecstasy (MDMA), Fentanyl (FEN, 5 ng/mL cutoff norfentanyl), Fentanyl III (FEN III, 1 ng/mL cutoff fentanyl), Hydrocodone (HYD), Methamphetamine (MAMP), Methadone II (MTD II), Methadone Metabolite (EDDP), Oxycodone (OXY III), Tramadol (TRAM), and FDA cleared De Novo Carisoprodol Metabolite (SOMA) Enzyme Immunoassays (EIAs).

In addition, LZI offers "Forensic Use Only" urinalysis reagents including Ethyl Glucuronide (EtG III), SPICE I (JWH-018), SPICE II (UR-144/XLR-II), and Ketamine (KET) EIAs.

We also provide a full panel of Homogeneous Oral Fluid Enzyme Immunoassay reagents including the FDA 510(k) cleared Oral Fluid 6-Acetylmorphine (OF 6AM), Amphetamine (OF AMP), Methamphetamine (OF MAMP), and THC (OF THC) EIAs, as well as "Forensic Use Only" Oral Fluid EIA reagents including Oral Fluid Cocaine Metabolite (OF COC), Ecstasy (OF MDMA), Methadone (OF MTD), Methadone Metabolite (OF EDDP), Opiate (OF OPI), and Oxycodone (OF OXY) EIAs.

We invite you to become our partners as we continue to develop cutting-edge technology in the world of clinical diagnostics.

EXHIBITORS

LNI SWISSGAS US LLC

Booth 604

50 Inwood Rd, Suite 104
Rocky Hill, CT 06067

Kim Georgiades

kgeorgiades@lniswissgasus.com

www.lni-swissgas.com

LNI is a multinational specialist in the manufacturing of premium gas generators for on-site hydrogen, zero air and nitrogen gas production, premium gas mixers and premium gas calibrators. LNI is ISO 9001 and ISO 14001 certified. To ensure the highest quality, LNI has received ISO 17025 accreditation of its Gas Flow Standard laboratory.

MEDIX BIOCHEMICA

Booth 207

www.medixbiochemica.com

Medix Biochemica is a market leading in vitro diagnostics raw material supplier with Finnish roots and global branches. We produce and supply high-quality antibodies, antigens, stabilizers, blockers and other critical IVD raw materials to enable our IVD customers to manufacture diagnostic tests and supporting materials all around the world. Our expertise covers market segments ranging from immunoassays, control materials, clinical chemistry and molecular diagnostics and capabilities include biospecimen accruals and contract manufacturing. With the most comprehensive raw material portfolio in the IVD world, and a team made up of the best minds in the business, we're ready to shorten your time to market and build quality into every one of your tests. Across disciplines and disease areas, whatever you need, chances are we IVDo that.

MERCEDES SCIENTIFIC

Booth 804

12210 Rangeland Parkway
Lakewood Ranch, Florida 34211

Stephanie Porter

Stephanie.Porter@mercedesscientific.com

mercedesscientific.com

Mercedes Scientific is the leader in laboratory supplies and equipment distribution with an unparalleled commitment to customer service.

EXHIBITORS

NEOGEN

Booth 702

Tier V Sponsor

Neogen® has been a trusted partner to the forensic toxicology and workplace drug testing industries for more than 30 years. We offer a broad range of immunoassays to detect drugs of abuse. Our comprehensive line includes an extensive range of ELISA kits that can be used to screen more than 300 drugs and their metabolites in various forensic matrices. Our solutions are designed for high throughput demands, so you can count on a cost-effective, optimized workflow without sacrificing accuracy or time.

We provide expert solutions, expert support, and exceptional results. At Neogen we don't just meet your drug-testing needs, we exceed them. Our team of experts is available to address your needs and determine how we can best support you.

NMS LABS

Booth 200

Tier III Sponsor

200 Welsh Road
Horsham, PA 19044

Cami Green

Cami.Green@nmslabs.com

www.nmslabs.com

NMS Labs delivers world-renowned forensic and clinical testing through an unmatched menu of toxicology and drug identification services. Backed by over 400 highly trained professionals, NMS Labs provides comprehensive panels and targeted testing options with industry-leading quality, scientific expertise, and rapid turnaround times to meet your most demanding needs. For more information visit: https://nmslabs.com/

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PHENOMENEX INC.

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Tier III Sponsor

Phenomenex is leader in products for forensic toxicology analysis. Our GC and LC columns and accessories, sample preparation consumables and application literature provide screening and confirmation solutions for drugs and metabolites in whole blood, urine, saliva, tissue and other complex matrices. Our scientists and technical experts are available for method development consultation, troubleshooting and training.

PHYTRONIX TECHNOLOGIES INC.

Booth 407

www.phytronix.com

The leader in quantitative ultra-fast high-throughput analysis solution for mass spectrometry presents the Axino Ion Source®. This sample introduction system and ionization source guarantees a quick, efficient and simple solution to your low-volume applications. Based on the patented LDTD technology, the Axino Ion Source® is the solution for forensics, toxicology and clinical applications.

RANDOX TOXICOLOGY

Booth 502

Tier III Sponsor

www.randoxtoxicology.com

With over 40 years of experience in the diagnostic market, Randox Toxicology is dedicated to advancing forensic, clinical, and workplace toxicology. Placing a heavy focus on new product R&D has led to the development of technology at the forefront of advanced global diagnostics. A market leader in the development of new assays and technology in the field of toxicology, Randox aims to minimise laboratory workflow constraints whilst maximising the scope of quality drug detection. With the world's largest toxicology test menu, screening for over 600 drugs and drug metabolites, our range of versatile analysers provides toxicology solutions for both high and low-throughput laboratories.

EXHIBITORS

RESTEK

Booth 806

Tier I Sponsor

Sean Kelley

sean.kelley@restek.com

www.restek.com

Chromatography is what Restek does, and chromatography is who we are. We are an independent, international, and diverse team of employee-owners not bound to a specific brand of instrument or geographic region. We live and breathe phase chemistry, peak separations, resolution, and inertness because while chromatography may be a necessary tool in your business, it is our business. And it is a business that we directly serve across 100+ countries and six continents with unrivaled Plus 1 service, applications, and expertise.

From LC and GC columns to sample preparation, reference standards to accessories, Restek is your first and best choice for chromatography.

SCIEX

Booth 500

Tier II Sponsor

https://sciex.com/applications/forensics-testing/forensic-toxicology

SCIEX is a global leader in mass spectrometry, delivering advanced forensic toxicology solutions through its powerful LC-MS/MS platforms. Our portfolio includes Triple Quad™, QTRAP®, and QTOF systems, enabling forensic laboratories to perform highly sensitive and specific drug screening and confirmation. SCIEX technologies support both targeted and non-targeted workflows, ideal for detecting known substances and identifying novel psychoactive substances (NPS) in complex biological matrices. With robust quantitation capabilities and comprehensive forensic spectral libraries, SCIEX systems streamline screen-to-confirmation processes, ensuring accurate, confident results. Whether analyzing dried blood spots or whole blood, SCIEX instruments offer exceptional performance, throughput, and reliability. Our innovative solutions empower forensic professionals to meet evolving analytical challenges with precision and efficiency, helping to modernize toxicology labs worldwide.

EXHIBITORS

SHIMADZU SCIENTIFIC INSTRUMENTS INC.

Booth 304

Tier I Sponsor

www.InvestigateYourLab.com

At Shimadzu, we have the analytical tools necessary for your forensic toxicology laboratory to be accurate, efficient, and confident with your results. Our products, including GC-MS, LC-MS/MS, UHPLC and ICP-MS, cover anything from sample preparation to screening, identification and confirmation. In addition, our wide range of instrumentation can be used with a variety of sample types, such as whole blood, urine, plasma, oral fluids, postmortem, tissues, etc.

SIEMENS HEALTHINEERS

Booth 505

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Tarrytown, NY 10591

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Siemens Healthineers is a leading medtech company with over 125 years of experience. We pioneer breakthroughs in healthcare. For everyone. Everywhere. Sustainably.

EXHIBITORS

TECAN

Booth 506

https://diagnostics.tecan.com/mass-spec-solutions-overview

Tecan's novel extraction cartridges and Resolvex® A200 system set new standards for automated sample preparation in clinical, forensic and toxicology laboratories. The Resolvex A200 offers walkaway automation for solid phase extraction (SPE) ensuring consistent, high-throughput processing of complex biological samples such as blood and urine. Tecan offers a range of universal extraction cartridges optimized for diverse analytes, supporting applications from therapeutic drug monitoring to forensic toxicology and clinical diagnostics. The system's intuitive interface and flexible protocol design enable easy adaptation to evolving laboratory needs, while minimizing manual handling and reducing the risk of cross-contamination. Combining robust hardware with high-quality consumables, Tecan empowers laboratories to achieve reproducible, reliable results - enhancing data quality and accelerating workflows in the most demanding analytical environments.

UCT

Booth 104

Tier I Sponsor

unitedchem.com

UCT is a manufacturer of high-quality sample prep products and rugged U/HPLC columns tailored for forensic applications. Explore the wide selection of Solid Phase Extraction phases, designed to accommodate a variety of application needs. Streamline extraction procedures by utilizing positive pressure manifold, glass-block manifold and SPeVAP® solvent evaporator. Obtain top-notch analytical precision in liquid chromatography with Selectra® U/HPLC columns and optimize chromatographic separations with SelectraCore® core-shell columns. Elevate gas chromatography analysis with SELECTRA-SIL® high purity derivatizing reagents and GC Liners. Simplify workflow with Select® pH buffer pouches and the Refine® ultra-filtration plates and columns. Visit us at www.unitedchem.com to find comprehensive forensic applications developed by our applications specialists. Learn about other SPE solutions by visiting our booth and engaging with the technical team.

EXHIBITORS

UTAK LABORATORIES, INC.

Booth 401

Tier III Sponsor

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Valencia, CA 91355

Christina Plutchak

christina@utak.com

www.utak.com

For over half a century, we have perfected the craft of manufacturing quality control (QC) materials for forensic laboratories.

We do not mass-produce generic solutions. We handcraft purpose-built QC materials tailored to your specific test methods, from routine drugs of abuse panels to the most challenging emerging compounds like fentanyl and xylazine.

Our process is simple: You specify the analytes, the matrix, and the target concentrations. We manufacture controls that align precisely with your assays, instrumentation, and case requirements.

The result is certainty. And forensic toxicology, certainty is not merely important, it is everything.

Control Made Simple.

WATERS CORPORATION

Booth 100

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Leading the way in Forensic Toxicology Solutions. Partner with Waters. Defend with Confidence.

Backed by decades of scientific expertise and a global support network, our robust instrument portfolio delivers reliable, high-quality data. Our platforms and workflows evolve with your laboratory's needs—supporting diverse applications and rapid response to emerging substances. From automated sample preparation to streamlined data processing, we help you overcome challenges and maximize throughput, so your team can focus on what matters most.

Learn more at waters.com/forensics

SHIMADZU

Excellence in Science

Powerful, Rugged Platforms for Forensic Toxicology Analysis

At Shimadzu, we have the analytical tools necessary for your forensic toxicology laboratory to be accurate, efficient, and confident with your results. Our products cover anything from sample preparation to screening, identification and confirmation. In addition, our wide range of instrumentation can be used with a variety of sample types, such as whole blood, urine, plasma, oral fluids, postmortem tissues, etc.

Ask about our solutions for:

- Drug Screening, Confirmation and Quantitation
- Heavy Metals Analysis
- Volatile Drug Screening and Confirmation
- Database Software Solutions for Identification
- High-Resolution Mass Spectrometry for Unknown Substance Analysis

Learn more at **Booth 304**

Shimadzu Scientific Instruments
7102 Riverwood Drive
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Leading the way in Forensic Toxicology Solutions. Partner with Waters. Defend with Confidence.

Backed by decades of scientific expertise and a global support network, our robust instrument portfolio delivers reliable, high-quality data. Our platforms and workflows evolve with your laboratory's needs—supporting diverse applications and rapid response to emerging substances. From automated sample preparation to streamlined data processing, we help you overcome challenges and maximize throughput, so your team can focus on what matters most.

To Learn More

Visit us in booth #100

waters.com/**forensics**

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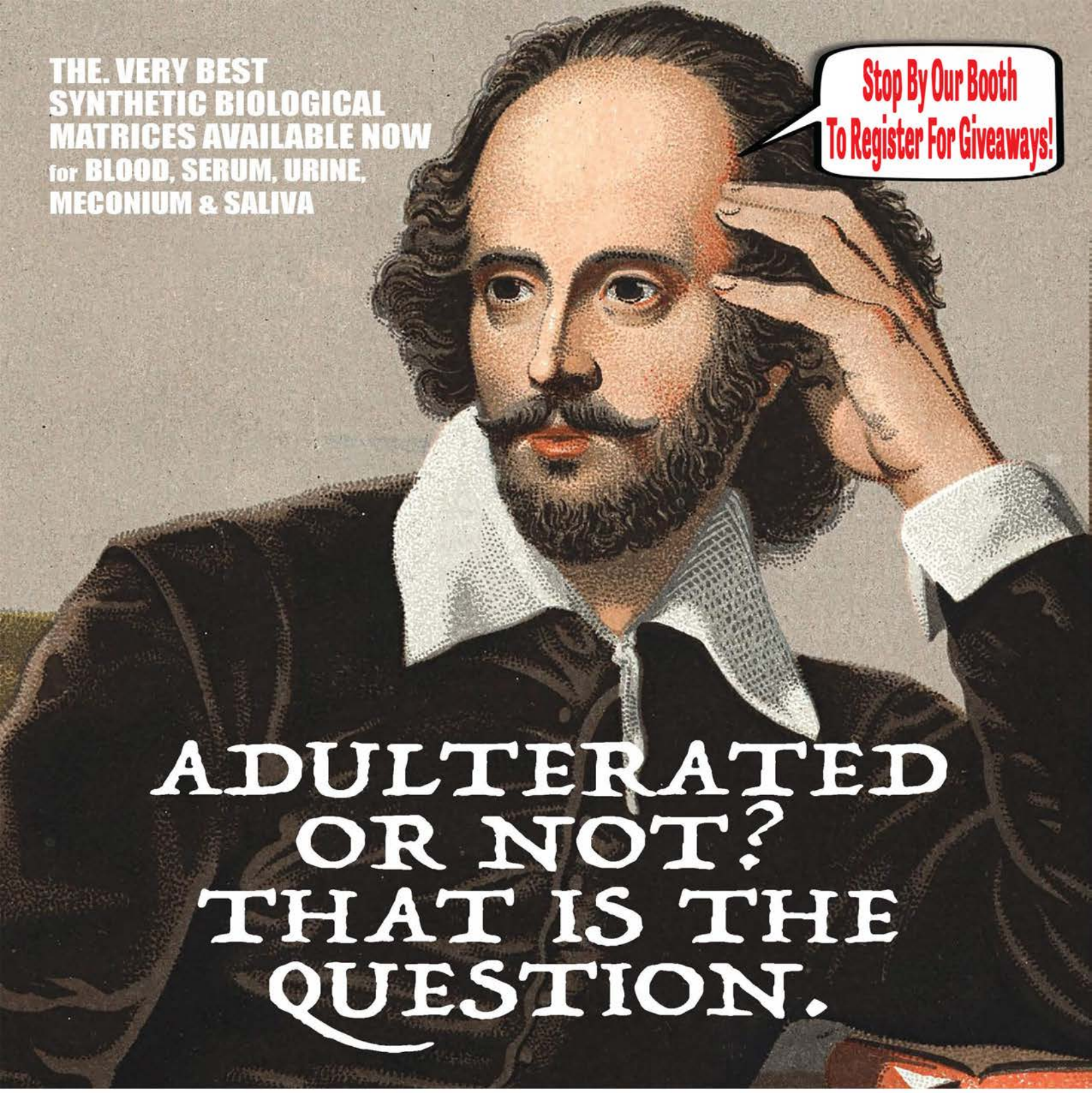

The Answer Is Axiom's SVTs!

Axiom's TEST TRUE liquid reagents provide superior quality at unbeatable prices. The TEST TRUE line includes assays for oxidants, nitrite, pH, specific gravity, chromate, creatinine, synthetic urine & the TRUETROL QA control set

Our QA Products including Refractometer Controls meet current SAMHSA guidelines

AXIOM Diagnostics™

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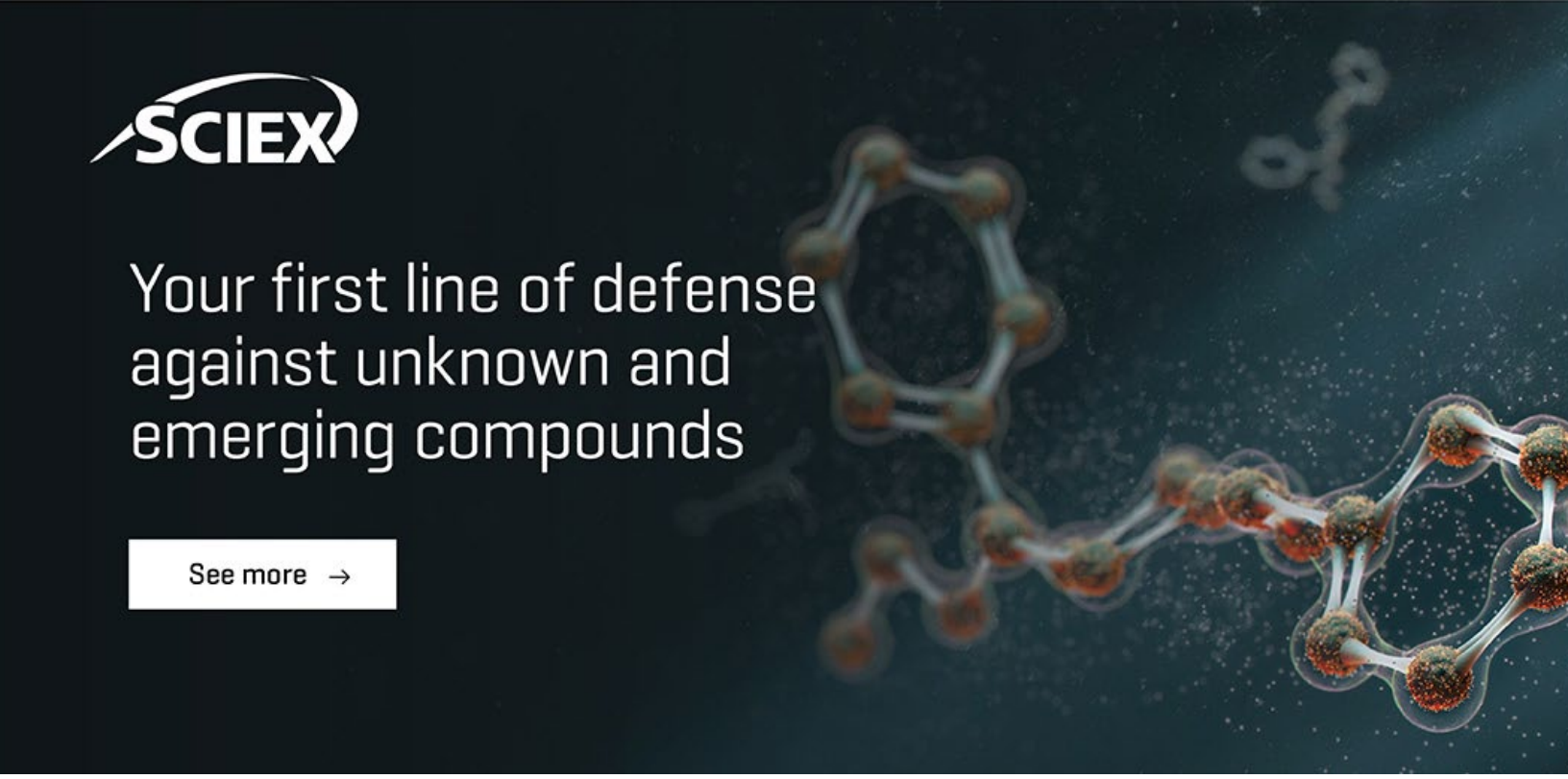

Stay ahead in forensic toxicology by seeing how others are using mass spec technology to detect and identify emerging threats. The SCIEX Forensic Resource Hub keeps you informed and connected and includes the following resources:

- **Tox Chat Box vodcast**
  Tune into this vodcast series highlighting how thought leaders leverage LC-MS/MS for cutting-edge forensic workflows.
- **Workshop presentations**
  Catch up on all the action and view videos from recent workshops at forensic conferences.
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FEN III

FENTANYL (Qualitative)

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510(k) Cleared

Lin-Zhi International, Inc. (LZI) Liquid Homogeneous Enzyme Immunoassay

Lin-Zhi International's LZI Fentanyl III Enzyme Immunoassay is intended for the qualitative determination of fentanyl in human urine at a cutoff value of 1 ng/mL when calibrated against fentanyl. Studies were performed on the Beckman Coulter AU5800. This product is CLIA-categorized as moderately complex on the Beckman Coulter AU480, AU680, and AU5800 analyzers.

Precision

Qualitative analysis: Typical results were measured in ΔOD, mAU

		Within-Run (N=22)		Total (N=88)	
	% of Cutoff	# Samples	EIA Result	# Samples	EIA Result
0 ng/mL	0%	22	22 Neg	88	88 Neg
0.25 ng/mL	25%	22	22 Neg	88	88 Neg
0.50 ng/mL	50%	22	22 Neg	88	88 Neg
0.75 ng/mL	75%	22	22 Neg	88	88 Neg
1.0 ng/mL	100%	22	22 Pos	88	4 Neg/84 Pos
1.25 ng/mL	125%	22	22 Pos	88	88 Pos
1.50 ng/mL	150%	22	22 Pos	88	88 Pos
1.75 ng/mL	175%	22	22 Pos	88	88 Pos
2.0 ng/mL	200%	22	22 Pos	88	88 Pos

Method Comparison

Qualitative Accuracy Study:

FEN Results 1 ng/mL Cutoff	Neg	< 50% below 50% of the cutoff concentration	Near Cutoff Negative between 50% below the cutoff and the cutoff concentration	Near Cutoff Positive between the cutoff and 50% above the cutoff concentration	High Positive greater than 50% above the cutoff concentration
Positive at or above the cutoff	0	20	12*	22	61
Negative below the cutoff	35	0	0	0	0

*** Discrepant samples contained levels of norfentanyl that contributed to the false positive result.**

Ordering Information

Ref #	Product	Size
0970	Fentanyl III EIA, Small Kit	(R_1/R_2) 100/37.5 mL
0971	Fentanyl III EIA, Large Kit	(R_1/R_2) 1000/375 mL
0973	Cutoff Calibrator: Contains 1 ng/mL fentanyl	5 mL
0977	Level 1 Control: Contains 0.5 ng/mL fentanyl	5 mL
0978	Level 2 Control: Contains 1.5 ng/mL fentanyl	5 mL

Fentanyl and Metabolite:

Compounds	Concentration Tested (ng/mL)	% Cross Reactivity	Result
Fentanyl	1.0	100.00%	Positive
Norfentanyl	2.5	40.00%	Positive

Cross Reactivity

Structurally Related Compounds

Compounds ND = Not Determined	Concentration Tested (ng/mL)	% Cross Reactivity
4-Fluoro-isobutyryl fentanyl	25	4.00%
9-Hydroxy risperidone	100,000	ND
Acetyl fentanyl	4	25.00%
Acetyl norfentanyl	100	1.00%
Acryl fentanyl	1	100.00%
Alfentanil	100,000	ND
Benzyl fentanyl	2	50.00%
Butyryl fentanyl	1	100.00%
Butyryl norfentanyl	40	2.50%
Carfentanil oxalate	100,000	ND
(±) cis-3-methyl fentanyl	4.5	22.22%
Cyclopropyl norfentanyl	15	6.67%
Despropionyl fentanyl (4-ANPP)	100,000	ND
Furanyl fentanyl	2.5	40.00%
Furanyl norfentanyl	150	0.67%
β-Hydroxyfentanyl	1.1	90.91%
(±)-β-Hydroxythiofentanyl	1	100.00%
Isobutyryl fentanyl	12.5	8.00%
Isobutyryl norfentanyl	390	0.26%
Methoxyacetyl fentanyl	1	100.00%
MT-45	100,000	ND
N-benzyl furanyl norfentanyl	0.75	133.33%
N-benzyl para-fluoro norfentanyl	1	100.00%
Norcarfentanil oxalate	100,000	ND
Ocfentanil	1	100.00%
Para-fluorobutyryl fentanyl	1.2	83.33%
Para-fluoro fentanyl	1	100.00%
Para-methoxy-butyryl fentanyl	1	100.00%
Remifentanil	100,000	ND
Risperidone	100,000	ND
Sufentanil	1,000	0.10%
Thienyl fentanyl	0.75	133.33%
Thiofentanyl	0.50	200.00%
Trans-3-methyl fentanyl	2.0	50.00%
Trazodone	100,000	ND
U-47700	100,000	ND
Valeryl fentanyl	50	2.00%
Ortho-methyl fentanyl	3.5	28.57%

DA: 10/07/2025

Lin-Zhi International, Inc.
2945 Oakmead Village Court
Santa Clara, CA 95051

ISO 13485:2016

SAL/0104-1.FEN III.Q.Rev.0

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