WELCOME TO COLORADO!

Home of the reigning NBA champions the Denver Nuggets, 58 peaks over 14,000 ft (or 4.3 km), and for one week, the Annual Society of Forensic Toxicologists Meeting. Whether you are a first-time attendee or have participated for many years, we hope this meeting exceeds your expectations for scientific content, networking, and fun! We are excited to welcome you to Denver, and hope you enjoy your time in this historic, lively, and beautiful Rocky Mountain city.

Our planning committee has worked diligently to plan a diverse and comprehensive week of scientific content. Unique to this year's meeting, Monday and Tuesday workshops, 16 to be exact, are all half-day workshops. The topics covered in the workshops include: pharmacology of synthetic drugs, addiction, method development and validation, pediatric toxicology, cannabis, measurement uncertainty, withdrawal, drug stability, novel psychoactive substances (NPS), drug facilitated crime (DFC), lean six sigma, oral fluid, and leadership. On Wednesday morning, the plenary speaker, Dr. Iris Wagstaff, STEM Program Director in the Diversity, Equity, and Inclusion Department of the American Association for the Advancement of Science (AAAS) will kick off the meeting. Scientific sessions will continue through Friday afternoon. Additionally, poster sessions on Wednesday and Thursday will take place in the Exhibit Hall, offering time to meet with the presenters, exhibitors, and new this year – puppies! Come by the puppy pen to destress and visit with the puppies.

In addition to outstanding scientific content, this year's meeting offers ample time to make new connections, and catch-up with old friends. The networking opportunities start on Sunday evening with the Young Forensic Toxicologists Symposium and continue through the week. Tuesday evening comprises the Welcome Reception with the exhibitors, the Elmer Gordon Forum, and the Nite Owl Reception. The off-site event on Wednesday evening will take place at Dry Dock North Brewery, a short drive away from the Gaylord Resort. This event will highlight Denver's craft-beer scene with locally brewed beer, food, games, and brewery tours. Enjoy the evening as you challenge your fellow scientist to an axe throwing or corn hole competition. Early Thursday morning, lace up your walking or running shoes for the Karla Moore Fun Run. Thursday evening, SOFT will recognize President Erin Spargo with a Masquerade Ball. Come dressed to impress and ready to dance to music from the multi-piece "Radio Band".

The talent of the planning committee combined with the knowledge of the SOFT Office ensured a successful planning process. Working alongside the SOFT 2023 planning committee has been an absolute pleasure. We would like to thank each of you for your collaboration, knowledge, and commitment for creating the best possible meeting. Thank you to the SOFT Office, Beth and CC for working through hotel logistics, schedules, meetings, and contracts – we've appreciated your expertise and guidance! Most importantly, we would like to thank all of you, the attendees, presenters, and exhibitors who make this meeting special every year.

We would be remiss if we did not take the opportunity to remember Toxicologist, friend, and Exhibitor Liaison Liz Kiely. Her untimely passing was felt by the entire planning committee. Liz served as Exhibitor Liaison for many past meetings, and this commitment will be recognized at the Liz Kiely Exhibit Hall. Please take some time this week to remember Liz and share your memories of her with others.

We are happy to welcome you to Denver. Be mindful of the altitude and stay hydrated (with water)! We hope everyone learns something novel, makes a new connection, and enjoys the Mile-High city!

- Dan and Vanessa

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The Late Liz Kiely

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Thank you to the many volunteers that have dedicated their time, expertise, and energy to ensure another successful SOFT meeting! With your help we have reviewed over 220 abstract submissions, restructured the workshop program to offer half-day workshops, our moderators will oversee nine platform sessions and two poster sessions, and another special issue of JAT has been created. We are grateful to each one of you and the many hours you have dedicated to SOFT and our community of toxicologists.

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SOCIETY OF FORENSIC TOXICOLOGISTS
YOUNG FORENSIC TOXICOLOGISTS

The Young Forensic Toxicologists (YFT) committee was founded in 2009 to promote education, networking, and interaction among young forensic toxicologists. This year the YFT committee will host the YFT Symposium, Professional Development Fair and The Student Enrichment Program. They will also select one Platform Winner and one Poster Winner for the Leo Dal Cortivo Award. The winners of the Leo Dal Cortivo Award will be announced at President Spargo's Masquerade Ball Thursday evening.

SYMPOSIUM AND PROFESSIONAL DEVELOPMENT FAIR
SUNDAY, OCTOBER 29TH, 5:00-9:00 PM

The Symposium begins with a social hour where hors d'oeuvres will be served and professional networking will be encouraged. During the Professional Development Fair (PDF), representatives from various accreditation/certifying agencies, graduate programs and laboratories will be available to discuss continuing education, professional training, board certification, resume and CV writing, and academic and career opportunities. Attendees must be 41 years of age or under to participate in the Symposium.

AGENDA

5:00 - 6:00 PM: Professional Development Fair with Resume/CV Review and Networking
6:00 - 6:10 PM: YFT Committee Introductions
6:10 - 6:25 PM: 2022 Leo Dal Cortivo Poster Award Winner - Joseph Kahl
6:25 - 6:35 PM: Olivia Skirnick - RTI and the NLCP
6:35 - 6:45 PM: SOFT Board Introductions
6:45 - 7:00 PM: Break
7:00 - 7:15 PM: Icebreaker
7:15 - 8:00 PM: Panel Speakers
8:00 - 9:00 PM: Panel Q&A / Open Forum Discussion

STUDENT ENRICHMENT PROGRAM
MONDAY, OCTOBER 30, 10:30 AM – 4:30 PM
(NOT OPEN TO SOFT ATTENDEES)

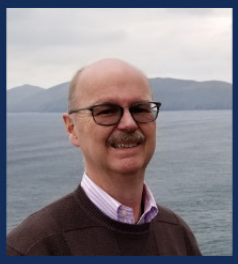
The Student Enrichment Program will be held Monday, October 30th and is **NOT** open to SOFT meeting attendees. This year YFT has partnered with the Colorado Bureau of Investigation and Aurora County Public Schools to welcome first and second-year forensic science students from Rangeview High School. The students will spend the day learning about forensic toxicology through presentations, virtual laboratory tours, and hands-on activities in partnership with SOFT's Diversity Task Force.

LEO DAL CORTIVO AWARD
THURSDAY, NOVEMBER 2, 7:00 – 8:30 PM

The Leo Dal Cortivo Memorial Fund allows the YFT committee to present two awards, each with a cash prize of $1,000 in addition to free registration at a future SOFT meeting. One award will be presented to the best poster presentation and the other for the best oral presentation. The 2023 winners will be announced at President Spargo's Masquerade Ball Thursday evening.

Employment opportunity board located by the registration desk: Bring your resume/CV and any open forensic toxicology positions to post for all attendees to see!

PANEL SPEAKERS



Dan Isenschmid

Certification

Dani Mata

Public Lab

Madeleine Swortwood

Professional Oranziations

Michelle Peace

Higher Education

Rachel Lieberman

Industry

LEO DAL CORTIVO

Joe Kahl

2022 Leo Dal Cortivo Winner

Panel Speaker Bios

Dr. Dan Isenschmid is a forensic toxicologist at NMS Labs near Philadelphia, Pennsylvania, USA. Prior to joining NMS he was the Chief Toxicologist at the Office of the Wayne County Medical Examiner (Detroit, Michigan) for over 17 years. Dr. Isenschmid received his Ph.D. degree in forensic toxicology from the University of Maryland at Baltimore, School of Medicine. He was the recipient of several Educational Research Awards from the Society of Forensic Toxicologists and the Irving Sunshine Award and Alexander O. Gettler Award from the Toxicology Section of the American Academy of Forensic Sciences.

Dr. Isenschmid is a Fellow and former Vice President of the American Board of Forensic Toxicology. He was the past Secretary and Treasurer of The International Association of Forensic Toxicologists and is a member and Past President of the Society of Forensic Toxicologists and a Fellow of the American Academy of Forensic Sciences where he also served on its Board of Directors. He has published and/or presented many papers on topics related to postmortem forensic drug testing including medical examiner case reports, the interpretation of postmortem cocaine concentrations, the stability and analysis of cocaine and its metabolites, and the effects of cocaine on human performance.

Dani C. Mata, MSFS, is currently a Senior Forensic Scientist for the Forensic Chemistry Bureau of the Orange County Crime Lab, CA where she has worked since 2007. Ms. Mata has been certified by the American Board of Forensic Toxicologists as a Diplomate in Forensic Toxicology since 2015 and is currently a Director on the board. In 2015 and 2016 Ms. Mata served as an affiliate on the Organization of Scientific Area Committee Task Group on Report and Testimony and starting in 2020 became a member of the Toxicology Subcommittee in 2020. Ms. Mata has been a member of the California Association of Toxicologists (CAT) since 2009 and has co-hosted two of the meetings and presented at nine CAT meetings. For CAT, she has co-chaired the Research Grant Committee for 2012 – 2014, and Literature Review Committee from 2012 – 2018. Ms. Mata has been a member of the Society of Forensic Toxicology (SOFT) since 2011 and has served on the Young Forensic Scientist Committee from 2013 – 2016, The Award Committee 2017 – 2020, The Toxicology Resource Committee 2020 to present and the Designer Drug Committee 2016 – present, where she has been the chair since 2017. She was the Volunteer co-Coordinator for the 2016 SOFT meeting and was the co-host to the SOFT 2020 meeting, which ended up being cancelled due to the COVID-19 pandemic. She was awarded the SOFT 2012 Young Scientist Meeting Award, SOFT 2021 Young Forensic Toxicologist Service Award and the 2022 AAFS Toxicology Section's Ray Abernethy Award for Outstanding Forensic Toxicology Practitioner.

Madeleine Swortwood, Ph.D. is currently a Toxicology Expert at Robson Forensic. Previously, she was a tenured Associate Professor in the Department of Forensic Science at Sam Houston State University. Dr. Swortwood received a Bachelor's degree in Biochemistry from Duquesne University and a Ph.D. in Chemistry from Florida International University (FIU). She completed a postdoctoral fellowship with the National Institute on Drug Abuse under the mentorship of Dr. Marilyn Huestis. During graduate school, Dr. Swortwood served as an intern at the Food and Drug Administration's Forensic Chemistry Center and the Miami-Dade Medical Examiner's Toxicology Laboratory, where she was hired as a full-time forensic toxicologist.

Madeleine is currently on the Board of Directors for SOFT and is also Co-Chair of the SOFT/AAFS Oral Fluid Committee. Previously, she served on the SOFT Publications Committee and the SOFT/AAFS Drugs & Driving Committee. She is past Chair of the AAFS Toxicology Section and serves as the Executive Secretary of the OSAC Toxicology Subcommittee. Her research to date includes novel psychoactive substances, oral fluid drug testing, drug metabolism, in utero drug exposure, and analytical method development. She has authored and co-authored more than forty manuscripts and one book chapter. She has mentored dozens of MS and PhD students while at SHSU and continues to chair dissertation committees.

Dr. Peace received her B.A. in Chemistry from Wittenberg University, a Master of Forensic Science from George Washington University, and her Ph.D. from the Medical College of Virginia at Virginia Commonwealth University (VCU).

Dr. Peace is a forensic toxicologist and a Full Professor in the FEPAC-accredited Department of Forensic Science at VCU and is one of the founding faculty for the Department. She served as Associate Chair and Chair for nearly a decade. Dr. Peace has also served as a manager in a private forensic drug testing laboratory and has worked as a scientist for Procter & Gamble, where she holds 3 patents.

Dr. Peace has been funded by the National Institute of Justice since 2014 to study the efficacy of electronic cigarettes, particularly as they pertain to substance use and abuse. Her research has highlighted emerging issues of electronic cigarettes as a tool for vaping drugs other than nicotine and has characterized the merging of the cannabis and e-cigarette industries. Her current project is a clinical study to assess the impact of vaping on roadside impairment evaluations for suspected DUI and drug testing.

Dr. Peace is a Past President of the Society of Forensic Toxicologists and is a member of The International Association of Forensic Toxicologists and is a Fellow in the American Academy of Forensic Sciences. She is a member of the National Safety Council's Alcohol, Drugs, and Impaired Driving Division. She was nationally recognized for Excellence in Teaching and Mentoring by the Society of Forensic Toxicologists.

Dr. Peace speaks regularly to train law enforcement and probation/parole officers, addiction specialists, attorneys, health system personnel, and primary and secondary education administrators on relevant issues regarding the mechanism of vaping and e-cigarettes as a tool to deliver drugs other than nicotine, as well as the effects of CBD and THC. She has provided testimony and opinions to develop scientifically relevant and robust policy and legislation at the state and federal levels, and she consults with companies and school systems as they re-develop smoking policies to include vaping.

Dr. Peace has testified to the Food and Drug Administration and the Virginia General Assembly regarding issues of quality assurance, public health, and public safety with the emerging cannabis industry. She has been featured in the New York Times, Consumer Reports, and AARP. The American Chemical Society and Discover Magazine recognized her and her team in 2018 and 2019 as some of the most influential research in the nation.

Rachel Lieberman received her B.S. in Chemistry with minor in Math from the University of Cincinnati and her Ph.D. in Analytical Chemistry from University of North Carolina– Chapel Hill under the supervision of Prof. James Jorgenson. Upon completing her studies in 2009, Rachel took a post-doc at National Institute of Standards and Technology from 2009 to 2011, working on standard reference materials. In May 2011, Rachel joined Shimadzu as an Application Scientist working on LC and LC-MS/MS products. In 2013, Rachel transitioned into the product group as a Sr. LCMS Product Specialist and was promoted to LCMS Product Coordinator. In October 2017, Rachel became the first Marketing Manager for Forensic Science at Shimadzu Scientific Instruments, and then took on the responsibilities of the Clinical market in October 2021. In April 2023, Rachel joined Waters Corporation as the Global Forensic and Toxicology Marketing Manager.

SUNDAY, OCTOBER 29

YFT Symposium and Professional Development Fair
5:00 - 9:00 pm
Open to attendees 41 years of age and younger

MONDAY, OCTOBER 30

Lunch & Learns with Sponsors
12:00 - 1:30 pm
Lunch & Learns are hosted by our generous SOFT Sponsors, check the app for additional information.

TUESDAY, OCTOBER 31

Lunch & Learns with Sponsors
12:00 - 1:30 pm
Lunch & Learns are hosted by our generous SOFT Sponsors, check the app for additional information.

Welcome Reception and Exhibit Hall Opening
6:30 - 9:30 pm
Visit all your favorite exhibitors while you peruse the hall. Food and beverage is provided.

Elmer Gordon Forum Dessert Reception
8:30 pm -10:00 pm
Moderated by SOFTopics, open to all attendees.

MilliporeSigma Nite Owl Reception
10:00 pm -12:00 am
The Nite Owl is hosted by MilliporeSigma and is open to all SOFT attendees.

WEDNESDAY, NOVEMBER 1

Exhibit Hall
9:30 am - 4:00 pm

Lunch with Exhibitors
12:00 - 2:00 pm

Lifeline Puppy Rescue
12:00 - 2:00 pm
Stop by and visit the puppies from Lifeline Puppy Rescue! Located in the Aurora Pre-function space.

Dry Dock Brewing - North
6:00 pm - 10:00 pm
Off-site Event, transportation is provided to and from the event. Food and beverage are provided at the venue.

THURSDAY, NOVEMBER 2

Karla Moore Fun Run/Walk
6:30 - 8:00 am
$30 Donation - Lapel pin and Shirt included, can be purchased at the check-in desk.

Exhibit Hall
9:30 am - 3:30 pm
Lunch with Exhibitors
12:00 - 2:00 pm

Lifeline Puppy Rescue
12:00 - 2:00 pm
Stop by and visit the puppies from Lifeline Puppy Rescue! Located in the Aurora Pre-function space.

SOFT Business Meeting
3:30 - 5:00 pm
Hear from the SOFT Board of Directors, officers will provide an update on the current status of SOFT. The ERA, YSMA, and Annual Awards are presented during the meeting. This event is open to all attendees.

President Spargo's Cocktail Hour
6:00 pm - 7:00 pm
Enjoy cocktails before the Masquerade Ball!

President Spargo's Masquerade Ball
7:00 pm - 12:00 am
Enjoy a plated dinner, drinks, and dancing! Masquerade masks are provided to attendees. The Leo Dal Cortivo Best Poster and Best Platform winners will be announced during the banquet. Meal card is required for entry.

THE KARLA MOORE TOX 'N PURGE FUN RUN/WALK

DR. KARLA MOORE

THURSDAY, NOVEMBER 2 FROM 6:30 - 8:00 AM

$30 DONATION - LAPEL PIN AND SHIRT INCLUDED

FUN RUN COORDINATOR: ARIA MCCALL

The original Tox 'N Purge run was created by Dr. Karla Moore in 1997 for the Salt Lake City meeting. In addition to her involvement in the field of toxicology and participation in SOFT, she was an officer in the United States Air Force. After her passing in 2008, the run was memorialized in her honor. The proceeds from the run are donated to the American Cancer Society in Dr. Moore's memory. Expenses for the event are supported by our SOFT Fun Run Sponsors.

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DR. IRIS R. WAGSTAFF
WEDNESDAY, NOVEMBER 1
8:00 - 9:00 AM
COLORADO B/C/D

Dr. Iris R. Wagstaff is a chemist, educator, mentor, researcher and science policy advisor. She currently serves as a STEM Program Director in the Inclusive STEM Ecosystems for Equity and Diversity (ISEED) Unit of AAAS where she manages a ~$30-million-dollar portfolio focused on broadening participation in STEM, inclusive workforce development, and advancing innovation capacity at HBCUs. In this role she also serves as Principal Investigator (PI) on several NSF-funded grants that support researchers and innovators from diverse backgrounds. She served as a 2015-2017 AAAS Science and Technology Policy Fellow at the Department of Justice (DOJ) National Institute of Justice (NIJ) Office of Investigative and Forensic Sciences where she developed and led an agency-wide DEI initiative.

She is a native of Goldsboro, NC with a BS and MS in Chemistry from UNC-Greensboro and NC A&T State Universities respectively; and a PhD in Science Education Research and Policy from North Carolina State University. She worked as a research chemist at the Dow Chemical Company for 15 years where she led analytical project teams and company-wide diversity initiatives. She has over 20 years of STEM outreach and advocacy developing strategic partnerships between industry, academia, and community organizations.

Iris is also a social scientist with a research focus on examining factors that predict science self-efficacy, science identity, and STEM career intent in K-12 underrepresented minorities (URMs). She is a thought leader and change agent in the broader STEM community. She serves on the Advisory Boards of several organizations that include the National Organization of Black Chemists and Chemical Engineers (NOBCChE), the American Chemical Society (ACS) Diversity, Equity, Inclusion and Respect board, and several NSF-funded grants. She is an adjunct chemistry professor at UNC-Greensboro where she leads DEI efforts in the chemical sciences. She is the Founder and Executive Director of Wagstaff STEM Solutions, an education and diversity consulting company. She has received several honors that include the 2020 DC Metro HBCU Alumni Alliance Education Award, the 2019 BEYA Science Trailblazer Award, and the 2018 NOBCChE Presidential Award for Mentoring, and the 2017 Women of Color in STEM Education Award.

Thank you to our 2023 ERA Donors, we are grateful for your support of this award and award recipients! Your generous donations total over $3,500 and will continue to support the Educational Research Award. Since its inception in 1980, the Educational Research Award has been presented to over 100 SOFT members for their research in forensic toxicology.

The purpose of the award is to encourage academic training and research in areas related to forensic toxicology and recognize worthy student researchers. The award is to be used to assist the awardee with travel expenses to attend the annual SOFT meeting for the purpose of presenting their research.

SOFT is a community of individuals that strive to support one another in their professional development and career growth. Many of our donors below are previous recipients of the ERA.

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Samantha Tolliver
Javier Velasco
Svante Vikingsson
Jeff Walterscheid
James H Watterson
Ruth Winecker

MONDAY, OCTOBER 30
8:00 AM - 12:00 PM

Workshop 1: Pharmacology, Detection, and Control Actions of Synthetic Drugs

- Chairs: Jonna Berry/Justin Grodnitzky
- Audience Knowledge: Intermediate

Workshop 2: The Human Brain and Factors Affecting the Neurobiological Development from Adolescent to Adulthood Exploring the Intersection of Mental Illness, Addiction, and the role of Laboratory Science

- Chairs: Michael Wagner/Tom Kupiec
- Audience Knowledge: Intermediate

Workshop 3: QTOF 101: A Guide to Successful Development and Validation (Part I)

- Chairs: Kaitlyn Palmquist/Christina R. Smith
- Audience Knowledge: Basic/Intermediate

Workshop 4: Pediatric Toxicology Part I: From Bottle to Backpack - Introduction to Pediatric Toxicology (Part I)

- Chairs: Jennifer Swatek/Kari Midthun
- Audience Knowledge: Basic

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

Workshop 5: Lessons Learned from Implementing QTOF Analysis into Routine Workflow (Part II)

- Chairs: Dani Mata/Brittany Casey
- Audience Knowledge: Basic/Intermediate

Workshop 6: Pediatric Toxicology Part II: From Bassinet to Body Bag - Postmortem Challenges and Considerations in the Investigative Process (Part II)

- Chairs: Jennifer Swatek/Kari Midthun
- Audience Knowledge: Intermediate

Workshop 7: Cannabis Testimony in Today's Environment

- Chairs: Jennifer Limoges/Stephanie Olofson
- Audience Knowledge: Intermediate

Workshop 8: beMUsed by Measurement Uncertainty? Let's Talk

- Chairs: Sue Pearring/Tate Yeatman
- Audience Knowledge: Basic

TUESDAY, OCTOBER 31
8:00 AM - 12:00 PM

Workshop 9: Win. Lose or Withdrawal: The Pharmacology, Management, and Interpretation of Drug Withdrawal

- Chairs: Aaron Shapiro/Nathalie Desrosiers
- Audience Knowledge: Advanced

Workshop 10: "Steady as She Goes": Mastering Stability in Forensic Toxicology

- Chairs: Karen Scott/Lorna Nisbet
- Audience Knowledge: Intermediate

Workshop 11: Forensic Interpretation of Novel Psychoactive Substances in Challenging Cases

- Chairs: Alex Krotulski/Dani Mata
- Audience Knowledge: Intermediate

Workshop 12: Drug-Facilitated Crimes (DFC) Analytical Methods and Statistics (Part I)

- Chairs: Laureen Marinetti/Celeste Wareing
- Audience Knowledge: Intermediate

TUESDAY, OCTOBER 31
1:30 - 5:30 PM

Workshop 13: Drug-Facilitated Crimes (DFC) Case Presentations (Part II)

- Chairs: Laureen Marinetti/Celeste Wareing
- Audience Knowledge: Intermediate

Workshop 14: Principles of Lean Six Sigma and Their Application to Forensic Toxicology Laboratories

- Chairs: Marissa Finkelstein/Joe Kahl
- Audience Knowledge: Basic

Workshop 15: Oral Fluid Testing: An Automatic Answer?

- Chairs: Robert Lockwood/Kristin Tidwell
- Audience Knowledge: Intermediate

Workshop 16: Career Development and Leadership Techniques for the Forensic Toxicologist

- Chairs: Kristin Kahl/Erin Strickland
- Audience Knowledge: Basic

PLATFORM PRESENTATION SCHEDULE

SESSION 1: CANNABIS

Wednesday, November 1

9:00-10:00 am

Location: Colorado B/C/D

Moderators: Marilyn Huestis & Cristina Sempio

Time	Platform Number	Title	Speaker
9:00-9:15 am	S1	Dose Effects of Oral And Vaporized Delta-8-THC and Comparison to Delta-9-THC in Healthy Adults	Austin Zamarripa
9:15-9:30 am	S2	Post-Collection Increase in Delta-9-THC Concentration is a Wisconsin Driver: A Case Study	Traci Reese
9:30-9:45 am	S3	Comparison of delta-9-tetrahydrocannabinol in venous and capillary blood following ad libitum cannabis smoking by occasional and daily users.	Gregory Dooley
9:45-10:00 am	S4	Meconium Marijuana Trends: Prevalence of Delta8-THCCOOH	Sarah Bartock
10:00-10:30 am - Morning Break			

SESSION 2: ALTERNATIVE MATRICES

Wednesday, November 1

10:30 am - 12:00 pm

Location: Colorado B/C/D

Moderators: Karen Scott & Curt Harper

Time	Platform Number	Title	Speaker
10:30-10:45 am	S5	Oral Fluid THC Results in a Green Lab Setting	Matthew Levitas
10:45-11:00 am	S6	The Detection of Cannabinoids in Exhaled Breath Condensate After Cannabis Use	Jennifer Berry
11:00-11:15 am	S7	Evaluation of Novel Psychoactive Substance Drug Loss from Storage in Serum Separator Tubes	Devin Kress
11:15-11:30 am	S8	The evaluation of alternative materials for a novel neonatal oral fluid collection device to monitor drug exposure	Ashley Gesseck-Harris
11:30-11:45 am	S9	Cocaine Hair Today, Gone Tomorrow: Unveiling the Impact of Cosmetic Treatments on External Contamination	Ana Miguel Fonseca Pego
11:45 am-12:00 pm	S10	Prevalence of Substance Use in US Court-ordered Mandatory Drug Testing of Oral Fluid and Hair	Jeri Ropero-Miller
12:00-2:00 pm - Lunch			

SESSION 3: POSTMORTEM

Wednesday, November 1

2:00-3:30 pm

Location: Colorado B/C/D

Moderators: Samantha Tolliver & Marissa J. Finkelstein

Time	Platform Number	Title	Speaker
2:00-2:15 pm	S11	Prevalence of foam cones in postmortem cases and the relationship with opioid-related deaths	Erin C. Strickland
2:15-2:30 pm	S12	Emergence of Fentanyl-Related Deaths in Travis County, Texas, and Surrounding Areas	Christina R. Smith
2:30-2:45 pm	S13	Bitter Brew: A Wake-Up Call on Caffeine-Induced Fatalities	Justin Brower
2:45-3:00 pm	S14	Household Harms: The Investigation of Deaths Caused by Household and Commercial Products	Marissa Finkelstein
3:00-3:15 pm	S15	The Importance of Toxicological Findings in a Mass Casualty Event	Laureen Marinetti
3:15-3:30 pm	S16	Investigation of postmortem formation of ethanol using ethyl sulphate and 1-propanol	Gerd Jakobsson
3:30-4:00 pm - Afternoon Break			

SESSION 4: CLINICAL

Wednesday, November 1

4:00-5:00 pm

Location: Colorado B/C/D

Moderators: Frank Peters & Marta Concheiro-Guisan

Time	Platform Number	Title	Speaker
4:00-4:15 pm	S17	Adverse events and mitragynine and 7-hydroxy-mitragynine plasma pharmacokinetics after controlled oral kratom leaf administration to healthy human participants	Marilyn Huestis
4:15-4:30 pm	S18	Assessing the Impact of Anabolic-Androgenic Steroids on Drug-Drug Interactions: In-Vitro Evaluation of Metabolic Clearance of Common Illicit and Medicinal Compounds	Richard L. Harries
4:30-4:45 pm	S19	High body mass index is prevalent in people with opioid use disorder in Northern England and increases risk of death	Caroline Copeland
4:45-5:00 pm	S20	Challenges with Fentanyl renewed use interpretation.	Erika Walker

SESSION 5: NOVEL PSYCHOACTIVE SUBSTANCES

Thursday, November 2

8:00-10:00 am

Location: Colorado B/C/D

Moderators: Simon Elliott & Sara Walton

Time	Platform Number	Title	Speaker
8:00-8:15 am	S21	Survey of Forensic Laboratories Testing for Novel Psychoactive Substances (NPS)	Alex Krotulski
8:15-8:30 am	S22	Prevalence of Novel Psychoactive Substances in Routine Prescription Drug Monitoring Clinical Urine Specimens	Jillian Neifeld
8:30-8:45 am	S23	Lipophilicity, plasma protein binding and intrinsic clearance of fentanyl analogues	Lorna Nisbet
8:45-9:00 am	S24	Novel "Pyrrolidino" Nitazene Analogues: The New Wave of Novel Synthetic Opioids Identified in Postmortem Cases	Sara E. Walton
9:00-9:15 am	S25	The Brothers Butonitazene: Connecting the Dots	Alexander Giachetti
9:15-9:30 am	S26	Emergence of the Novel Opioid N-Desethyl Isotonitazene in the Recreational Drug Supply	Joshua DeBord
9:30-9:45 am	S27	Fluorexetamine and 2-fluoro-2-oxo-phenylcyclohexylethylamine: New Dissociative Hallucinogens in Forensic Toxicology and Drug Chemistry Casework	Danai T. Taruvinga
9:45-10:00 am	S28	First Identification of (±)-cis-Δ8- and Δ9-Tetrahydrocannabinol in Biological Specimens by One-Dimensional UHPLC–MS/MS	Szabolcs Sofalvi
10:00-10:30 am - Morning Break			

SESSION 6: ANALYTICAL METHODS

Thursday, November 2

10:30 am - 12:00 pm

Location: Colorado B/C/D

Moderators: Jochen Beyer & Sara Dempsey

Time	Platform Number	Title	Speaker
10:30-10:45 am	S29	Previously Unidentified Major Hydroxylated Metabolite of Cannabidiol	Svante Vikingsson
10:45-11:00 am	S30	OSAC Forensic Toxicology Subcommittee Development of a Standard Test Method for Ethanol in Blood	Rebecca Wagner
11:00-11:15 am	S31	LC-MS/MS Quantification of Δ8-THC, Δ9-THC, THCV Isomers and Their Main Metabolites in Human Plasma	Cristina Sempio

11:15-11:30 am	S32	Prenatal Exposure to Kratom: Development of an LC-MS/MS Assay for Mitragynine and Speciociliatine in Umbilical Cord and Comparison with an Assay for Mitragynine in Meconium	Melissa Hughs
11:30-11:45 am	S33	Performance of an Untargeted LC-HRMS Screening Method for Tier I Substances	Heather Barkholtz
11:45 am-12:00 pm	S34	Development of an Automated Sample Preparation Method	Tyler Devincenzi
12:00-2:00 pm - Lunch			

SESSION 7: AWARDS

Thursday, November 2

2:00-3:30 pm

Location: Colorado B/C/D

Moderators: Sara Schreiber & Luke N. Rodda

Time	Platform Number	Title	Speaker
2:00-2:15 pm	S35	Assessing the Cross-Reactivity of Cannabinoid Analogs (Delta-8 THC, Delta-10 THC and CBD) and their Major Metabolites in Six Commercial Cannabinoid Urine Screening Kits	Ashley Pokhai
2:15-2:30 pm	S36	Analysis of over 250 Novel Synthetic Opioids by LC-MS/MS in Blood and Urine	Katie Diekhans
2:30-2:45 pm	S37	Impact of Blood Preservatives and Anticoagulants on Cannabinoid Quantitative Analysis	Bridget O'Leary
2:45-3:00 pm	S38	Application of Liquid Chromatography Quadrupole Time-Of-Flight Mass Spectrometry (LC-QTOF-MS) All Ions Data Analysis to Authentic Blood Specimens	Jessica Ayala
3:00-3:30 pm - Afternoon Break			

SESSION 8: DRUGS AND DRIVING

Friday, November 3

8:00-10:00 am

Location: Colorado B/C/D

Moderators: Rebecca L. Hartman & Nathalie Desrosiers

Time	Platform Number	Title	Speaker
8:00-8:15 am	S39	Combining toxicology testing with FST results to improve cannabis impairment classification	Robert L. Fitzgerald
8:15-8:30 am	S40	Evaluation of the field sobriety tests in identifying drivers under the influence of cannabis	Thomas Marcotte

8:30-8:45 am	S41	Acute and chronic oral dosing of cannabidiol (CBD) with and without low doses of delta-9-tetrahydrocannabinol (Δ-9-THC)	Ryan Vandrey
8:45-9:00 am	S42	The Society of Forensic Toxicologists' Toxicology Resource Committee 2023 Survey Results	Sabra Jones
9:00-9:15 am	S43	Interference of the novel designer benzodiazepine 4'-chloro deschloroalprazolam with alprazolam analysis in toxicology and seized drug DUID casework	Corissa Rodgers
9:15-9:30 am	S44	Impact of drug-driving legislation in England & Wales on fatal road traffic collisions following drug use	Caroline Copeland
9:30-9:45 am	S45	The Prevalence of Drugs in Motor Vehicle Fatalities in Jefferson County, AL (2017-2022).	Karen S Scott
9:45-10:00 am	S46	Fentanyl in DUID Cases - A Comparison of Three Regions: Alabama, Orange County, CA, and Houston, TX	Curt Harper
10:00-10:30 am - Morning Break			

SESSION 9: BEST PRACTICES

Friday, November 3

10:30 am - 12:00 pm

Location: Colorado B/C/D

Moderators: Sumandeep Rana & Sue Pearring

Time	Platform Number	Title	Speaker
10:30-10:45 am	S47	Results of a Trial Program for a 32-Hour Work Week in a Forensic Toxicology Laboratory	Matthew Juhascik
10:45-11:00 am	S48	An Update on Standards Development Activities in Forensic Toxicology	Marc LeBeau
11:00-11:15 am	S49	Implementation of Consensus-based Standards Made Easier	Laurel Farrell
11:15-11:30 am	S50	Underreported methamphetamine positives by laboratories following SAMHSA urine drug test reporting guidelines.	Sumandeep Rana
11:30-11:45 am	S51	Is Chat GPT capable of producing court- ready interpretations of toxicology reports ?	Kathleen Rice Davies
11:45 am-12:00 pm	S52	Finding Forensic Evidence: Toxicological Results from Drug-Facilitated Sexual Assault Cases in Eastern Denmark from 2015–2022	Kathrine Skov

POSTER PRESENTATION SCHEDULE

POSTER SESSION #1
Wednesday, November 1, 12:00-2:00 pm
Location: The Liz Kiely Exhibit Hall
Moderators: Heather L. Ciallella & Jaime Lintemoot

Poster Number	Title	Speaker
P1	Evaluation of the Correlation between LC/TOF Drug Screening and GC-MS Confirmation Results for Cannabinoids at the Indiana State Department of Toxicology	Alexander San Nicolas
P2	Development of a Novel Xylazine ELISA for the Rapid Screening of Xylazine in Human Whole Blood and Urine Samples	Pamela Greiss
P3	DoD Cholinesterase Monitoring Program and Detection of Acute Organophosphate Poisoning	Pucheng Ke
P4	Quantitation of 106 drugs in urine using a fast, 7-minute method with high resolution accurate-mass (HRAM) mass spectrometry	Courtney Patterson
P5	Development of a Bead Ruptor-based method for rapid toxicological analysis of synthetic opioids in bones	Michaela Gysbers
P6	In vivo Toxicity Evaluation of the Russian Homemade Drug Krokodil	Emanuele Alves
P7	Fatality due to combined toxicity of Xywav® and ethanol	Anna Kelly
P8	Confirmation of Cannabinoids in Forensic Toxicology Casework by Isomer-Selective UPLC-MS-MS Analysis in Urine	Thomas Rosano
P9	Design and Distribution of Traceable Opioid Material (TOM) Kits to Improve Laboratory Testing for Opioids and Associated Drugs of Concern	Rebekah Wharton
P10	Whole Blood Drug Panel Screening in Impaired Driving Investigations: Development of a Rapid Screening Method using Laser Diode Thermal Desorption coupled to mass spectrometer (LDTD-MS/MS).	Serge Auger
P11	Biomarker, prescribed and illicit polar drug class analysis in urine: Development of a rapid screening method using LDTD-MS/MS.	Serge Auger
P12	Desalkylgidazepam: the new kid on the block in British Columbia	Sandrine Mérette
P13	An Unusual Suicide by Benzonatate Overdose: A Case Report Utilizing Method of Standard Addition to Confirm Benzonatate Toxicity	Elizabeth A. Taylor
P14	PFAS in Me: Which Ones and How Much?	Karl Oetjen
P15	The 'Spice' of Life: Identifying a Common Source in a Series of MDMB-4en-PINACA Deaths and a Human Performance Case	Kei Osawa
P16	Sensitivity improvement for the detection of steroid hormones and endocannabinoids in keratinized matrices	Pierre Negri
P17	Targeted and non-targeted analysis of fentanyl analogs and their potential metabolites using LC-QTOF	Leonard Chay

P18	New hydrolysis and analysis for screening SAMHSA Drug Panel in urine: Development of a rapid screening method using LDTD-MS/MS.	José Luis Callejas
P19	Determination of four diamide-insecticides in blood by liquid chromatography-tandem mass spectrometry: Application to a case of cyantraniliprole poisoning	Sungmin Moon
P20	Ensuring Consumer Health: Diagnostic Ion Screening for Illicit Substances and Prescription Drug Detection in Gas Station Supplements	Karl Oetjen
P21	Simultaneous determination of mescaline, mitragynine, 7-hydroxymitragynine and psilocin in urine samples by liquid chromatography-tandem mass spectrometry	Meejung Park
P22	Comparison of Hydrolysis Efficiency and Performance of Four Recombinant β-glucuronidase Enzymes for the Detection of Opioids in Urine Samples by LC-MS/MS	Marta Concheiro
P23	Validation of an Analytical Method for Quantitation of Metonitazene and Isotonitazene in Plasma, Blood, Urine, Liver, and Brain and Application to Authentic Casework in New York City	Justine Pardi
P24	Head(space) to Head(space): Volatile Analysis by Gas Chromatography Mass Spectrometry/ Flame Ionization Detector (GC-MS/FID) and Gas Chromatography Dual FID	Allison McBride
P25	1,1-Difluoroethane (DFE) Prevalence in Kansas	Kayla Smith
P26	Δ8-THC Impact on Non-Regulated Marijuana Confirmation Testing Rates	David Kuntz
P27	Separation of 11-Hydroxy-THC Metabolites and Quantitation of 18 Total Cannabinoids in Whole Blood by UHPLC-MS/MS	Melissa Beals
P28	Determination of 18 Cannabinoids in Urine with Separation of 11-OH-THC Metabolites by UHPLC-MS/MS	Michael Clark
P29	Column Selectivity Screening for Analysis of a Panel of Negative Mode Illicit Drugs in Urine by LC-MS/MS Using “Dilute and Shoot”	Shahana Huq
P30	Testing DUID Casework for all Tier One Drugs: A 18-month Review	Tyler Devincenzi
P31	Prevalence of Xylazine and Fentanyl in Umbilical Cord Specimens from High Risk Populations in the United States	Dominique Gidron
P32	Simultaneous determination of Δ8-THCCOOH and Δ9-THCCOOH in hair by LC-MS/MS	Meejung Park
P33	LC-QTOF-MS Method Development and Validation for the Screening of Nitazene Analogs in Whole Blood	Amanda Pacana
P34	LC/MS/MS analysis of 11-nor-9-carboxy-tetrahydrocannabinol in fingernails	Juseon Lee
P35	Best Practices: Hydrolysis Protocols Are Not a “One Size Fits All” for Each Drug Class, when using B-One	Janet Jones
P36	Urine Drug Surveillance in Philadelphia, PA, with Emphasis on Xylazine and its Metabolites	Alyssa Reyes
P37	The Evolving Landscape of THC Drug Testing, Delta-8 vs. Delta-9	Jamie York

P38	Demographic differences in drug-related deaths	Thikra Algahtani
P39	The Emergency of Bromazolam in Jefferson County, Alabama: A Case Series	Lisa M. Bianco
P40	Death by Depressants – a Case Study in Drug and Ethanol Postmortem Distribution	Jennifer Jimenez
P41	Toxicological profile of diquat and bromide ion concentrations in blood in a fatal poisoning case	Maiko Kusano
P42	Analysis of Drug-infused Papers by ASAP-MS	Emily Lee
P43	Analysis of carboxy-THC in hair using UPLC-MS/MS	Jane Cooper
P44	Comparison of Data-Independent and Data-Dependent Techniques for Forensic Toxicology Screening Analysis	Jonathan Danaceau
P45	Urinary Forensic Toxicology Data Independent Analysis Screening: Using High Resolving Power Multi-Reflecting Time-of-Flight Mass Spectrometry	Jonathan Danaceau
P46	Evaluating the stability of promethazine in solution with the addition of hydrochloric acid	Kelsey Cooper
P47	Pediatric Deaths: A 10-year review of cases handled by Onondaga County Medical Examiner's Forensic Toxicology Laboratory.	Kristie Barba
P48	Evaluating Methcathinone and Pseudoephedrine Levels in Over-the-Counter Drugs and Corresponding Urine Samples	Te-I Weng
P49	The Benefits of 2.1 mm Internal Diameter Analytical Columns for the Analysis of Drugs of Abuse by LC-MS/MS	Samantha Herbick
P50	Determining the optimum derivatizing agents and parameters to improve the detection of anabolic-androgenic steroids	Richard L. Harries
P51	Investigations into the Human Metabolism of the Minor Cannabinoid Cannabichromene	Alexandra Ward
P52	Evaluation of a Quantitative Analysis Method for Tetrahydrocannabinol Isomers in Biological Matrices	Rebecca Wagner
P53	Validation of traditional and designer benzodiazepines in blood by gas chromatography-tandem mass spectrometry (GC-MS/MS)	Zhenqian Zhu
P54	Detection of the Substituted Cathinone Alpha-PiHP in Postmortem Toxicology Cases	Kevin G. Shanks
P55	Prevalence of tramadol and its metabolites in the Orange County DUID population over a 6-year period	Nancy Kedzierski
P56	Sevoflurane and Ethanol - How Hospital Treatment for an Injury Can Cause Big Problems in a Standard Alcohol Case	Amy Rutgers
P57	Chemicals in Urine Can Reduce Glucuronide Hydrolysis Efficiency Causing False Negatives for Drug of Abuse Analysis	Amanda McGee
P58	Benzonatate: Cures acute cough, causes analytical headache.	Sandra Bishop-Freeman

P59	The Rising Prevalence of Fentanyl With Methamphetamine in DUID Cases	Helen Chang
P60	Using a Virtual Liquid Chromatography Tool to Develop Methods for Novel Psychoactive Substances	Haley Berkland
PMP	Benefits and Impact of the Professional Mentoring Program to SOFT	Amanda Cadau

POSTER SESSION #2
Thursday, November 2, 12:00-2:00 pm
Location: The Liz Kiely Exhibit Hall
Moderator: Helen Chang & Lexi May

Poster Number	Title	Speaker
P61	Development and Validation of a Comprehensive Dilute-and-Shoot LC-MS/MS Method: Qualitative Confirmation of 113 Drugs in Urine	Taylor Hunt
P62	Simplified Workflow for Whole Blood Testing in Medicolegal Death and Impaired Driving Cases	Jeffery H. Moran
P63	Streamlined LC-MS/MS Workflows for Drug Facilitated Crime Investigations	Jeffery H. Moran
P65	A Comparison of the Performance of LC-MS/MS Analytical Methods for Workplace Drugs of Abuse Testing Using Zero-Grade Air and Nitrogen Gas	Martin Jacques
P66	Trends in Suicides and Drug Involvement: Exploring the Impact of the Pandemic in the Forensic Toxicology Landscape	Jeanna Mapeli
P67	Evaluation of Sample Ionization Sources for the Characterization of Isomeric Fentanyl Analogs Utilizing Trapped Ion Mobility Spectrometry Time of Flight Mass Spectrometry (TIMS-ToF MS).	Samuel Miller
P68	Development of SEFRIA Ecstasy Oral Fluid Assay Meeting SAMHSA Guideline	Guohong Wang
P69	Forensic identification of tobacco products: method development and optimization	Pierre-Yves Martin
P70	Validation of a Simple Chromatographic Method to Screen Oral Fluid Samples for Tier I Drugs in DUID Investigations	Gregory Sarris
P71	Human Factors May Influence THC Capture with an Impaction Filter Device: Towards Standardized Sampling and Calibration Protocols	Kavita Jeerage
P72	Withdrawn	
P73	Reference concentrations and distribution of 7-aminoclonazepam in hair following a single dose of clonazepam	Marie Katrine Klose Nielsen
P74	Immobilized Enzymes on Magnetic Beads for Separate Mass Spectrometric Investigation of Human Phase II Metabolite Classes	Ioanna Tsiara
P75	Identification of “Nitazenes” in whole blood samples by LC-MS/MS and LC-HRMS/MS	Adhly Huertas-Rivera

P76	In Vitro Formation of Hydroxy Metabolites in Hair after Hair Product Exposure	Nichole Bynum
P77	Long-term Volatile Stability in Whole Blood Containing Different Additives and Assessment for Isobutylene Interference in BD Vacutainer® Tubes	Haley Melbourn
P78	Prevalence of opioid use among gabapentin positive urine specimens	Lixia Chen
P79	Evaluation of a fast and novel workflow for urine toxicology screening with DART-MSMS and Toxbox®	Francois Espourteille
P80	Rapid and Sensitive Quantification of Psilocin in Human Whole Blood using Liquid Chromatography Tandem Mass Spectrometry (LC-MS/MS)	Munchelou Gomonit
P81	It's Not Just Fentanyl Anymore: Implications for Drug Delivery Resulting in Death Cases	Sherri Kacinko
P82	What is the Preferred Educational Background of Forensic Scientists?	Heather Barkholtz
P83	Withdrawn	
P84	A non-evaporative HLB dispersive pipette extraction (DPX) for sensitive analysis of therapeutic and abused drugs in urine	Madison K. Webb
P85	Systematic Web Monitoring of Drug Test Subversion Strategies in the United States	Megan Grabenauer
P86	Modification of Specimen Validity Testing Ranges in Non-Regulated Urine Testing	Brittany DeWitt
P87	Separation of Delta-8, Delta-9 THC and Chiral Hexahydrocannabinol metabolites in urine from Pain Management Compliance Testing	Carl E. Wolf
P88	Fentanyl and Acetyl Fentanyl Concentrations in Postmortem Blood Specimens over a Six Year Period	Megan Savage
P89	Fentanyl Overdoses and Use of Randox MultiSTAT as an Investigative Tool in Postmortem Toxicology	Clarissa Lawen
P90	Rapid, Simple, and Cost-Effective Postmortem Quantification of β-Hydroxybutyrate in Whole Blood Using the Abbot Precision Xtra® Point of Care Device	Camille Colletti
P91	Trends in Pediatric Intoxication Deaths in Maryland State Over a Twenty Year Period (2003 -2022)	Rebecca Phipps
P92	Prevalence of Opioid Glucuronides in Human Hair	Katherine Bollinger
P93	Running from a Truth: Lack of Regulation Promotes CBD in Health-conscious Markets Without Oversight	Kimberly Karin
P94	An Advanced Method for Detecting Fentanyl, Fentalogues, Xylazine and Nitazenes by UHPLC-MS/MS	Kimberly Yacoub
P95	Detection of Phosphatidylethanol (PEth) in 12–15 Month-Old Whole Blood Specimens	Justin L. Poklis
P96	National Laboratory Certification Program Studies in the Preparation of Hair PT Samples	E. Dale Hart

P97	4-Year Evaluation of Drug Impaired Driving Drug Concentrations	Grace Cieri
P98	Towards Determination of Recent Cannabis Use: Optimizing Cannabinoid Recovery from an Impaction Filter Device	Mary Gregg
P99	Designer Benzodiazepines in Alabama Over a 5-Year Period	Mary Ellen Mai
P100	Drug Evaluation and Classification (DEC) cases in Ontario: A 2-year review of regional trends and toxicology results	James Rajotte
P101	A Postmortem Case Report Involving Desalkylgidazepam and Bromazolam	Leena Dhoble
P102	Analysis of Xylazine, Opioids, and Other Common Adulterants in Blood and Urine by SPE and LC-MS/MS	Stephanie Reichardt
P103	Review of first year LC-QTOF-MS screening of blood specimens from suspected DUID cases for NPS Benzodiazepines	Wayne Lewallen
P104	Analysis of Δ8 and Δ9 THC Metabolites and Other Cannabinoids by SPE and LC-MS/MS	Emily Eng
P105	Screening and Confirmatory Testing of a Counterfeit M-30 Pill Adulterated with Xylazine	Michael Wakefield
P106	2-Methyl AP-237: A Case Series involving a Novel Synthetic Opioid	Kristopher W. Graf
P107	LC-MS/MS-based Quantification of 47 Therapeutic Drug Monitoring Compounds in Serum: A Simple Sample Preparation Strategy for Efficient Analysis	Scott Krepich
P108	The use of Cocaine Hydroxy Metabolites in a Decision Model for the Interpretation of Ingestion vs. Exposure in ChildGuard® Samples	Andre Sukta
P109	The emergence and implications of altered codeine content in poppy seeds used in the food industry	Heather Hambright
P110	Development and Validation of an LC-MS-MS Method for the Quantitative Analysis of Ten Cannabinoids	Elsayed Ibrahim
P111	Sensitive and accurate characterization of Z drugs, metabolites and their degradation profile in urine using LC-MS/MS	Holly Pagnotta
P112	Phenibut Concentrations in a Fatality Quantitated using Method of Standard Addition	Andrea Edel
P113	Stability of Ethanol in Electronic Cigarette Liquid Formulations	Alaina Holt
P114	Determining Cannabinoid Acetate Analog (delta 9-THC-O-A, delta 8-THC-O-A, and CBD-di-O-A) Metabolites Using Rodent Brains	Natalie Ortiz
P115	Evaluating the Impact of Expiration and Additives on Blood Ethanol Concentration Stability in Vacutainer® Gray Stopper Tubes	Amanda Green
P116	Translating a Standard Curve between Drugs as a Proof of Concept to Quantify a Drug and its Metabolite in a Single Sample	Katherine Gussenhoven
P117	A Statistical Comparison of Fentanyl and 4-ANPP in the Postmortem Population of Washington, DC	Hunter Fleming

P118	Miniaturization Strategies for Streamlined Drugs of Abuse Extraction prior to UH-PLC-MS/MS Analysis	Lee Williams
P119	Harmonizing Out the Variability of an LC-MS/MS Instrument Fleet	Ali Saleh
P120	Comprehensive novel psychoactive substance (NPS) and synthetic opioids screening in dried blood spots (DBS) using HRMS	Marc Browning
P121	NPS Surveillance through High Resolution Mass Spectrometry Screening of Forensic Samples: Insights and 2022 Trends	Donna Papsun
P122	Prevalence of Ketamine in Hair and Nails in High-Risk Populations	Liaqat Abbas
P123	Prevalence of Fluorofentanyl and Designer Opioids in Alabama 2021-2023	Karen Valencia
P124	Automated Detection and Re-Analysis of Over-Range and Saturating Samples	Matthew Campbell
P125	The Difference a Glucuronide Can Make: Comparing the Stability of Psilocin vs Psilocin-glucuronide	Gregory Janis
P126	Evaluating the efficacy of commercial oral fluid collection devices	Ashley Gesseck-Harris

S01

Dose Effects of Oral and Vaporized Delta-8-THC and Comparison to Delta-9-THC in Healthy Adults

Austin Zamarripa[1], Ashley Dowd[1], Tory Spindle[1], Elise Weets[1], Edward Cone[1], Ruth Winecker[2], Ron Flegel[3], David Kuntz[4], Ryan Vandrey[1]

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Abstract

Introduction: There has been a proliferation of retail cannabis products in the U.S. predominantly containing delta-8-tetrahydrocannabinol (delta-8-THC) as a result of the U.S. Agriculture Improvement Act of 2018. However, little controlled research on delta-8-THC has been conducted since the 1970s.

Objective: The goal of the present study was to characterize the acute effects of oral and vaporized delta-8-THC, compared with a positive control dose of delta-9-THC and placebo, on subjective drug effects, cardiovascular effects, cognitive performance, and pharmacokinetics.

Methods: Healthy adults (N=13) were recruited to participate in 2 studies. The first study evaluated the acute effects of oral delta-8-THC (0 mg, 10 mg, 20 mg and 40 mg) and oral delta-9-THC (20 mg) infused within a chocolate brownie. The second study evaluated the acute effects of delta-8-THC (0 mg, 10 mg, 20 mg and 40 mg) and delta-9-THC (20 mg) administered via vaporization. The experiments were run concurrently with most participants opting to complete both studies in sequential order. In those cases, the order of study completion (i.e., oral then vaporized or vice versa) was counterbalanced. Both studies used a within-subject crossover design, dose order within each study was randomized, and a minimum of 6 days separated each test session to allow for drug washout. Vital signs, self-reported drug effects, performance on a battery of cognitive tasks, and blood, urine and oral fluid samples were obtained from participants at baseline and repeatedly for 8 hours after dosing.

Results: To date, 11 of 13 enrolled participants have completed the oral dosing study and 10 of 20 have completed the vaporization study. In the oral dosing study, pharmacodynamic assessments show a dose-orderly effect of delta-8-THC on all assessments. The 20 mg delta-9-THC dose showed qualitatively stronger drug effects than all 3 doses of delta-8-THC on most measures, though the 40 mg delta-8-THC dose was comparable. Consistent with the pharmacodynamic data, delta-8-THC conditions produced dose orderly increases in maximum concentrations of delta-8-THC and its metabolites in blood, oral fluid, and urine. In the vaporized dose study, pharmacodynamic outcomes showed stronger peak drug effects compared with the oral dose study, which is consistent with prior cannabis research. There was a lack of dose-orderliness on subjective drug effects for delta-8-THC in the vaporized dose study as many outcomes were rated similarly across the 3 doses of delta-8-THC. Puff topography data showed that puff volume was lower for the 20 mg and 40 mg dose conditions compared with the 10 mg dose and placebo, suggesting participants were titrating inhalation behavior. Delta-9-THC produced qualitatively higher effects on several subjective drug effect outcomes compared with delta-8-THC, but the 40 mg delta-8-THC dose produced similar or stronger effects on heart rate and cognitive performance compared with 20 mg delta-9-THC in this study. Delta-8-THC produced dose-orderly increases in concentrations of delta-8-THC and its metabolites in blood and urine, but not oral fluid, following active delta-8-THC conditions.

Discussion: Acute doses of oral and vaporized delta-8-THC produced pharmacodynamic drug effects that fully overlap in distinguishing characteristics with the acute effects of delta-9-THC. However, effects of delta-8-THC were lower in magnitude than effects produced by the same dose of delta-9-THC. This is consistent with prior research on binding affinity between the 2 isomers. The 40 mg dose of delta-8-THC resulted in equivalence or near equivalence in the magnitude of drug effect observed at the 20 mg dose of delta-9-THC on most outcomes.

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S02

Post-Collection Increase in Delta-9-THC Concentration is a Wisconsin Driver: A Case Study

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Abstract

Introduction: Marijuana (cannabis) refers to the dried leaves, flowers, stems, and seeds of the Cannabis sativa (C. sativa) plant and is the most commonly abused federally scheduled illicit drug in the United States. The primary psychoactive compound present in C. sativa is delta-9-tetrahydrocannabinol (delta-9-THC), which is self-administered for its mood-altering ability. In the State of Wisconsin, delta-9-THC is a Schedule I restricted controlled substance (RCS) and operating a vehicle with any detectable amount in whole blood is a violation of the law. A 25-year-old male was observed by an officer driving at a rate of speed that appeared greater than the 25 mile per hour (MPH) speed limit. When the officer made contact with the driver, he noted the odor of marijuana. Evidence suggests that the subject ingested an unknown amount of dried cannabis plant material after his initial contact with the officer. After performing Standardized Field Sobriety Tests (SFSTs), the officer placed the driver under arrest for Operating While Intoxicated (OWI). The driver was transported to a hospital where a phlebotomist drew the subject's blood into two gray-top vacutainer tubes containing 100 mg of sodium fluoride and 20 mg of potassium oxalate. These tubes were forwarded to the Wisconsin State Laboratory of Hygiene (WSLH) for toxicology testing.

Objectives: This case study examines an unusually high delta-9-THC concentration and its subsequent increase documented through repeated testing. The author highlights a gap in the literature regarding oral administration of cannabis products and examines the abnormal observation of Horizontal Gaze Nystagmus (HGN).

Methods: All specimens received by the WSLH undergo ethanol and other volatiles analysis. A comprehensive drug screen was performed through lipid/matrix removal cleanup with detection by Waters Acquity UPLC I-Class and Waters Xevo G2-XS Quadrupole Time of Flight (QToF) Mass Spectrometer (Waters Corp., Milford, MA, USA), which was positive for delta-9-THC metabolites. Confirmatory, quantitative analysis for delta-9-THC and its metabolites was performed using SLE cleanup with Biotage Isolute SLE+ 96-well plates (Biotage Corp., Uppsala, Sweden) and detection by a Shimadzu Prominence Liquid Chromatograph (Shimadzu Corp., Kyoto, Japan) and Applied Biosystems 4000 Tandem Mass Spectrometer (Applied Biosystems, Waltham, MA, USA). Limit of quantitation (LOQ) is 1.0 ng/mL for delta-9-THC. The linear dynamic range (LDR) for delta-9-THC is 1.0 ng/mL-50.0 ng/mL.

Results: The WSLH reported a delta-9-THC concentration of 320 ng/mL. Subsequent tests over a year-long period have shown increasing concentrations of delta-9-THC. The most recent measurements show that the delta-9-THC concentration has increased by more than 150% from the original reported value.

Discussion: Most studies evaluate the effects of inhaled THC, as it is the most common route of administration due to its quick onset of action. However, as the legal market for cannabis expands, a wide range of delta-9-THC-containing products that may be orally ingested ("edibles") have become available. There is a notable lack of scientific literature that examines the effect of oral administration of dried plant material on blood delta-9-THC concentrations. In the raw plant material, the non-psychoactive precursor to delta-9-THC is present as a monocarboxylic acid, tetrahydrocannabinolic acid (THCA-A). It is well documented that THCA-A is converted to delta-9-THC through a thermally-mediated pathway. Studies have shown that this conversion is incomplete. Additionally, THCA-A may be detected in the urine and blood of cannabis users. THC impairment is well documented. The officer reported observation of HGN, a symptom of impairment not typically associated with THC. The author postulates that the observed oculomotor phenomenon represents a THC-mediated change in saccadic eye movements. To the author's knowledge, 320 ng/mL in whole blood is the highest reported concentration of delta-9-THC observed in a driver.

S03

Comparison of delta-9-tetrahydrocannabinol in venous and capillary blood following ad libitum cannabis smoking by occasional and daily users.

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Abstract

Introduction: In forensic investigation of transportation crashes or workplace mishaps requireing blood THC determination, venipuncture by a phlebotomist is often the standard blood collection protocol. Blood THC concentrations decline quickly due to tissue distribution, so the longer time passes until collection, the less the blood measurement may reflect that at the time of the incident. Automated collection of capillary blood at the scene of transportation or workplace incidents using self-contained commercially available collection devices may facilitate a more rapid approach to forensic blood collection.

Objective: To perform the first systematic comparison of venous and capillary THC concentrations obtained from smokers of contemporary potency cannabis.

Methods: Thirty healthy adults (aged 25-55) were recruited into three groups (n = 10 each): occasional use (approximately 1 – 12 times a month) of cannabis flower (bud) (15 – 30% total THC by weight), daily use of flower products; and daily use of cannabis concentrate products (60 to 90 % total THC). Participants inhaled their own cannabis products with dispensary labeled THC concentrations ad libitum during a 15-minute interval following instructions to consume "the amount you most commonly use for the effect you most commonly desire." Matched time point venous blood samples were collected via an in-dwelling venous catheter, and lateral proximal upper extremity (shoulder skin) capillary samples were obtained with the TAP-II device at baseline and 10 minutes after the last inhalation, and 30, 60, 90, and 145 minutes after the start of the inhalation period. The TAP-II device uses suction via a microneedle array to collect 100 to 250 µL capillary samples directly into tubes containing an anticoagulant. Samples were frozen immediately after collection until time of analysis. THC concentration in both venous and capillary blood were determined using LC-MS/MS with LOQ of 0.5 ng/ml. THC measurements less than LOQ were assigned a value of zero. Absolute difference in blood THC (capillary – venous; ng/mL) were compared with the Wilcoxin Rank Sum test.

Results: Venous samples were collected at all timepoints (n = 180). Collection of capillary samples was successful in 135 of 192 attempts (70 %), with failures resulting from inadequate volume collection (< 50 µL) and from the device (adhesive or skin puncture failures). Capillary blood THC concentration was consistently lower than venous blood THC at all time points after cannabis inhalation. The magnitude of the difference was greatest at the earliest post inhalation time point and diminished thereafter. Median venous whole blood THC concentration 10 minutes after last inhalation were 10.0, 61.7, and 62.8 ng/mL for occasional flower, daily flower, and daily concentrate users, respectively. The corresponding median capillary blood THC values were 7.2, 31.0, and 34.2 ng/mL, respectively. At 60 minutes after the initiation of inhalation, median venous whole blood THC had declined to 1.2, 10.5, and 14.8 ng/mL for occasional flower, daily flower, and daily concentrate users, respectively. The respective median capillary THC values at 60 minutes were 0.7, 2.9, and 9.6 ng/mL, representing decrements relative to venous blood of 42%, 72%, and 35%. Comparisons for other time points were also calculated.

Discussion: Capillary blood THC concentration sampled with the TAP-II device at the shoulder underestimated concurrently measured venous whole blood THC, often by considerable percentages, in the minutes to hours after cannabis inhalation. This may have forensic implications if results were compared to per se or permissible inference of intoxication cut-points in some jurisdictions. Collection efficiency was suboptimal, even following controlled research protocols. Future investigation of THC in capillary blood might explore refinements in methodology, and the impact of sample collection, as pharmacokinetics may be influenced by cutaneous capillary density and other site-specific factors.

PLATFORM

S04

Meconium Marijuana Trends: Prevalence of Delta8-THCCOOH

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Abstract

Introduction: Marijuana is the most used federally illicit substance, although many states have legalized medical and nonmedical use for adults. The 2017 Pregnancy Risk Assessment Monitoring System showed 4.2% of pregnant women self-reported marijuana use during pregnancy, with as high as 14.8% among women 20-24 years old. Prenatal cannabis exposure may be associated with lower birth weights and gestational age, as well as poorer intelligence test performance and increased depressive symptoms when the exposed neonates reach school-age. With the 2018 legalization of hemp, Δ9-tetrahydrocannabinol (Δ9-THC) isomers, such as Δ8-tetrahydrocannabinol (Δ8-THC), have grown in popularity with minimal oversight and differing legal statuses. With lower potency than Δ9-THC, Δ8-THC is perceived as safer than Δ9-THC, while still producing similar desirable effects. Currently, no information on Δ8-THC use among pregnant women is available.

Objectives: Evaluate marijuana positivity in meconium, the first neonatal fecal sample passed after birth, by reviewing screening and confirmatory test results for Δ9-THCCOOH, the primary Δ9-THC metabolite observed in meconium, and to estimate the prevalence of Δ8-THCCOOH in meconium specimens.

Methods: Limited data sets from 7,224 consecutive meconium specimens submitted from across the United States to a screen-to-confirmation workflow, and from 315 meconium specimens that proceeded directly to confirmation testing, were assessed for cannabinoid positivity.

A Randox ELISA kit on a Hamilton Starlet with a Biotek PowerWave plate reader was used for marijuana screening (Δ9-THCCOOH cutoff 20ng/mL). Confirmatory meconium marijuana testing involved an ANSI/ASB-validated laboratory-developed, liquid chromatography tandem mass spectrometry (LC-MS/MS) assay to quantify Δ9-THCCOOH between 2.5 and 5,000ng/mL. Meconium concentrations were adjusted to ng/g by meconium weight with a 5ng/g cutoff. Quality controls verified Δ8-THCCOOH relative retention time, abundance, and that no conversion of 7-COOH-cannabidiol to Δ9-THCCOOH occurred. Cannabinoid isomers were resolved on a Luna Omega Polar C18 100×2.1mm, 3µm column (Phenomenex, Torrance, CA). Δ9-THCCOOH was detected in negative mode using a 4500 Sciex triple quadrupole mass spectrometer.

Results: Δ9-THCCOOH positivity was confirmed by LC-MS/MS in 882 (12.2%) screen-to-confirmation specimens with a concentration median (mean, range) of 140ng/mL (220ng/mL, 6-3900ng/mL). Other specimens that screened positive for cannabinoids had quantities insufficient for confirmation (n=51), were confirmed negative (n=12), or were released with an unable to confirm message related to interference observations (n=18). Of the 315 meconium specimens that proceeded directly to confirmation testing, 258 (81.9%) had Δ9-THCCOOH confirmed with a concentration median of 60ng/mL (138ng/mL, 5-2000ng/mL). Other specimens confirmed negative (n=55) or were released with an interference message (n=2).

Confirmatory LC-MS/MS data were reviewed from a subset of 280 consecutive meconium specimens, including screened-positives and direct-to-confirm specimens, to determine the prevalence of Δ8-THCCOOH. Of these, Δ8-THCCOOH was detected in 61 (21.8%). Compared with Δ9-THCCOOH, 35 (12.5%) had lower and 26 (9.3%) had higher Δ8-THCCOOH concentrations. Of the latter, 9 were released as Δ9-THCCOOH negative and 11 were released with the interference message (where the higher Δ8-THCCOOH caused poor chromatographic resolution on a low-positive Δ9-THCCOOH). The overall Δ8-THCCOOH meconium prevalence within screen-to-confirmation orders was estimated at 3.2% by extrapolating the subset period positive frequency to the entire study period, corrected for number of direct-to-confirmation specimens used during the period (4.3%).

Discussion: Within the cohort, we observed Δ9-THCCOOH positivity in about 13% of screened and 12% of confirmed specimens with an average Δ8-THCCOOH prevalence of about 3%. These prevalence estimates may prompt physicians

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and investigators to enquire about prenatal Δ8-THC use and request reporting on cannabinoid isomers in these important, irreplaceable specimens. The study limitations may include for-cause ordering bias; however, the study population was nationally based, and some hospital systems have a random or universal testing approach additionally imposed.

S05

Oral Fluid THC Results in a Green Lab Setting

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Abstract

Introduction: 'Green Labs' are law enforcement trainings that give police officers hands-on experience with an individual who has recently used cannabis. Officers encounter drivers who have used cannabis and administer psychophysical tests to determine if the subject is impaired. Unlike alcohol, many officers receive little training to determine if a subject is under the influence of a drug. Green Labs help address this training gap for cannabis.

Objectives: Forensic Fluids Laboratory (FFL) partnered with law enforcement entities conducting Green Labs to collect oral fluid specimens to evaluate the change in delta-9-THC (THC) concentrations over time after consumption. FFL also sought to compare oral fluid toxicology results with Drug Recognition Expert (DRE) observations.

Methods: Green Lab subjects (consumers) used their own cannabis product(s) in two consumption sessions (15 to 30 minutes long and separated by 90 to 120 minutes). Each consumer was paired with a DRE for the duration of the Green Lab. The DRE's collected oral fluid samples from the consumers at set time points. For each consumer, samples were collected prior to cannabis consumption, 10 and 30 minutes after the first consumption session, 10 and 30 minutes after the second consumption session, and before the conclusion of the Green Lab. All oral fluid samples were screened by ELISA for delta-9-THC and delta-8-THC (Cutoff = 1 ng/mL), and a panel of common drugs of abuse. All samples were automatically tested for delta-9-THC, delta-8-THC, and CBD using LC-MS/MS (LOQ's = 0.5 ng/mL). Samples that screened positive for other drugs were confirmed by LC-MS/MS.

Results: Oral fluid samples were collected from 33 consumers across three Green Lab training sessions. Table 1 shows THC concentrations ranges measured before and during consumption. Individual THC time-course concentrations over time were plotted for each consumer (demographics and graphs not shown due to abstract limitations). Concentrations over time after consumption varied between consumers. DRE results for each consumer were shared from two of the three Green Labs.

Pre-Consumption (ng/mL)	Post-Consumption Maximum (ng/mL)	Post-Consumption Minimum (ng/mL)
0.0 – 77.3	3.6 – 4817.0	0.5 – 368.2

Discussion: The concentration of cannabis product and amount consumed were not the same. Consumers brought their own cannabis products, so there was no uniformity in THC concentrations. Additionally, they ingested by their own preference – leading to differences in the amount consumed between individuals. The consumers did provide us with the THC concentrations from the product packaging and stated how much they consumed, but this information may not be entirely accurate, nor verifiable.

Consumers used different cannabis formulations. We observed consumers vaping concentrates, smoking joints, and ingesting edibles or using tinctures. Consumers who vaped or smoked reached maximum oral fluid THC concentrations within 30 minutes of use. Mucosal deposition was suspected to be a factor based on the magnitude of concentrations observed between consumers. In contrast, consumers who ingested edibles showed increasing oral fluid THC concentrations over the time course of collections.

There was no relationship between the THC concentrations measured in oral fluid and the determination of 'impairment' by the DRE's. For example: one consumer with THC a concentration less than 5 ng/mL was deemed impaired, while another consumer at 77 ng/mL was deemed not impaired.

Though confounding, these variables cannot and should not be strictly controlled. In authentic impaired driving cases, it is unlikely that much of this information would be available. For this reason, the collections we facilitated serve as an additional source of information to better understand oral fluid THC concentrations. In the future we intend to modify our collection scheme, test more edible-only consumers, and document issues with collection device saturation.

S06

The Detection of Cannabinoids in Exhaled Breath Condensate After Cannabis Use

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Abstract

Introduction: The legalization and decriminalization of recreational cannabis use across the United States has increased the need for roadside detection of cannabis use during the window of impairment. Uncertainty remains about the relationship of Δ9-tetrahydrocannabinol (THC) levels in biological matrices to impairment, so current research has focused on determining recent use. THC is theorized to be carried in exhaled breath aerosols and several published studies have investigated offline analysis of aerosols captured with filters. Multiple breaths are required to produce picogram to nanogram device concentrations. Exhaled breath condensate (EBC) is a rich matrix that contains both aerosols and volatile organic compounds and has yet to be explored in the context of cannabis use.

Objectives: In this pilot study, the primary objective is to analyze EBC collected with a commercial device and identify compounds associated with recent cannabis use.

Methods: EBC is collected by chilling exhaled breath to condense water vapor and volatile organic compounds. Aerosols are captured by sedimentation. The collection tube is cooled with a metal collar stored at -80 °C. The participants are asked to breathe deeply through the device, with a breathing maneuver to increase breath aerosols, for 5 min while their breaths are counted. The three cannabis use groups include participants who smoke cannabis daily, participants who vape concentrates daily, and participants who smoke cannabis less than daily. Participants provide their own legal-market products and there is also a non-use control group. EBC is collected before cannabis use (baseline) and at two time points (~1 h and ~2 h) after monitored cannabis use (the control group relaxes during this time). EBC is stored in the collection tube at -80 °C until it is extracted and processed for analyses. EBC is concentrated to dryness by lyophilization and reconstituted in solvent with deuterated cannabinoid internal standards. Analytes are then separated on an ultra-high performance liquid chromatography system with triple quadrupole tandem mass spectrometry (UHPLC-QQQ-MS/MS) to detect and quantify THC and 10 other cannabinoids, if present. Multiple reaction monitoring of two transitions from each of the 11 cannabinoids (THC-COOH, THCV, CBD, CBG, CBG-A, CBN, Δ9-THC, Δ8-THC, Δ10-THC, CBC, THC-A) and 10 internal standards (Δ10-THC did not have a matched internal standard) allows for stringent identification criteria for all analytes.

Results: While this is an ongoing study, the first batch of samples that includes 16 total participants with four participants from each group has been analyzed. In all samples from participants who used cannabis, THC was detected in the first (1 h) post-use sample. THC decreased or was not detected in the second (2 h) post-use sample. THC was detected in some baseline samples, despite a requested 8 h abstinence before the study session. Cannabinol (CBN), cannabigerol (CBG), tetrahydrocannabinolic acid (THCA), and tetrahydrocannabivarian (THCV) were detected in some – but not all – post-use samples.

Discussion: THC was recovered from the aqueous condensate matrix in all 1 h post-use samples studied to date and some 2 h post-use samples. THC was not detected in any of the samples from the control group, demonstrating that handling protocols effectively prevent cross-contamination. In post-use samples, four different cannabinoids were detected in addition to THC; detection of one or more of these compounds could potentially improve the reliability of breath-based determinations of recent use. The results described here are preliminary and represent a small portion of the larger study (90 total participants anticipated in the cannabis use groups) with an intent to understand the viability of the EBC matrix as a means to capture cannabinoids in breath following cannabis use.

PLATFORM

S07

Evaluation of Novel Psychoactive Substance Drug Loss from Storage in Serum Separator Tubes

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Abstract

Introduction: Serum separator tubes (SST) are a type of blood collection tube used primarily for clinical testing. After testing is completed, it is common practice to store remaining serum in a SST. A concern that arises with this practice is certain drugs are susceptible to adsorption into the polymer gel that separates the serum and red blood cells, which can result in a decreased concentration of drug in the serum. This is problematic for forensic toxicology as the lower concentration of the drug can affect interpretation. This phenomenon has been investigated with some drugs, but information on the impact of storage in SST on novel psychoactive substances (NPS) is limited.

Objectives: The aim of this study is to determine if statistically significant loss of NPS opioids (nitazenes), NPS stimulants, and NPS benzodiazepines occurs during storage in SST for 14, 60, and 90 days in refrigerated conditions, respectively.

Methods: A control was prepared in citrated human whole blood. 5 mL of the control was then aliquoted into a 5 mL gold-top Becton Dickinson Vacutainer SST. The blood was re-calcified with 37.6 µL of 2M calcium chloride, inverted six times, left undisturbed for 30 minutes to clot, and centrifuged at 3000 RPM for 10 minutes. 1.5 mL of serum was transferred to a borosilicate glass tube (GT) and the remaining serum was left in the SST. These aliquots were stored refrigerated (4°C) and analyzed on days 0, 1, 2, 7, 14, 30, 60, and 90.

Results: Relative percent difference in concentration over the time period and two tailed, two-sample paired variance t-test ($p<0.05$) were calculated from the resulting mean of each test day for the SST and GT controls.

NPS Benzodiazepines:

The percent change in concentration from day 0 to day 90 in the SST and GT ranged from -42% to -1.5% and -26% to 0%, respectively. By day 90, five of the seven NPS benzodiazepines had a statistically significant (t-test, $p<0.05$) decrease in concentration by day 90. Flubromazolam and 8-aminoclonazolam were unaffected by storage in the SST. Clonazolam showed a 32% decrease in concentration when stored in the SST, however, it showed a similar decrease in the GT (26%) suggesting a stability issue of the compound rather than loss of the drug into the gel polymer in the SST.

NPS Stimulants:

All seven NPS stimulants demonstrated a statistically significant decrease in concentration when the sample was stored in the SST. The percent change in concentration from day 0 to day 60 in the SST ranged from -65% to -23%, respectively, while the percent change in the GT from day 0 to day 60 ranged from -13% to +1.3%, respectively. Dimethylpentylone suffered the largest loss in concentration while being stored in the SST while showing almost no difference in concentration between day 0 and 60 when stored in GT.

NPS Opioids (Nitazenes):

All six NPS opioids displayed a statistically significant decrease by day 2. The percent change in concentration from day 0 to day 14 in the SST and GT ranged from -88% to -51% and -3.5% to +29%, respectively. Testing will continue to day 90 to determine if this trend continues.

Discussion: There was significant loss of basic drugs into the gel polymer, and in some cases this could cause the concentration to fall below the limit of detection. In cases where blood is collected and stored in a SST, the best practice is to decant the serum as soon as possible into another tube. In the event that quantitative testing is performed on serum from a SST, the possibility of drug loss should be considered in any interpretation.

PLATFORM

S08

The evaluation of alternative materials for a novel neonatal oral fluid collection device to monitor drug exposure

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Abstract

Introduction: Oral fluid may be a more desirable matrix to monitor neonatal drug exposure due to the invasiveness, challenges, and limitations of collection, and/or analytical difficulties of traditional matrices. Traditional oral fluid collection devices are not viable options for neonates due to the large size and potential chemical transfer. Unstimulated and stimulated infant saliva, whole saliva, and oral fluid samples have been used as a diagnostic tool for detection of systemic diseases, monitoring of drugs, pollutants, electrolytes, and hormones. Oral fluid samples have been collected by both commercially available and ad hoc devices. Careful consideration must be taken for the type of fluid collected as well as process of collection for any diagnostic testing to be useful.

Objectives: To assess the suitability of swabs and alternative materials (SAMs) for neonatal oral fluid collection by evaluating recovery and stability of sixteen drugs and/or metabolites in lab-created oral fluid.

Methods: Eight commercially available swabs, five commonly available alternative materials, and three commercially available oral fluid collection devices were evaluated for absorptive properties, analyte recovery, and stability. The absorptive properties of SAMs and collection devices were completed by saturating the absorbent materials with water and comparing the difference in dry versus wet weight. The initial recovery study was evaluated by saturating each material with fortified water. Seven SAMs were selected to assess recovery from lab-created oral fluid. Five swabs and no alternative materials were selected to compare to commercially available collection devices for recovery and stability of 2-Ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine, 6-acetylmorphine, 11-nor-9-carboxy-THC, amphetamine, benzoylecgonine, buprenorphine, cocaine, codeine, cotinine, delta-9-tetrahydrocannabinol, fentanyl, methamphetamine, methadone, morphine, norbuprenorphine, and norfentanyl. A lab-created oral fluid (SOF) was fortified with 200 ng/mL of each analyte. Swabs and buffer-less devices were saturated with SOF and devices containing buffer had 1.0 mL SOF quantitatively transferred to the collection pad. To mimic an authentic collection, SOF was allowed to absorb to the collection pad for 5 minutes prior to placing the pad into the buffer. Swabs were centrifuged to remove the oral fluid from the absorbent material, devices were treated per manufacturer recommendations. Analyte recovery was evaluated by comparing the concentration of drug obtained from each swab and device to a neat oral fluid specimen in triplicate by a previously validated liquid chromatography with tandem mass spectrometry method. Stability was evaluated at room, refrigerated, and freezer temperatures in triplicate.

Results: The absorptive properties of the materials ranged from 2 – 150 times their dry weight in water. The commercial devices produced 3 – 13 of the 16 analytes with ≥80% recovery and 9 – 15 with ≥50% recovery from the collection pad. The swabs produced 1 – 10 of the 16 analytes with ≥80% recovery and 4 – 15 with ≥50% recovery from the material.

Discussion: The recovery of analytes varied between SAMs and commercially available oral fluid collection devices. The use of oral fluid for diagnostic testing and drug monitoring will be limited by the absorbent material used for collection and the analyte(s) of interest. Ad hoc collection devices from commercially available swabs may be inadequate and studies are needed to evaluate their limitations. The need for a smaller, sterile, easy-to-use, oral fluid collection device is imperative to non-invasive diagnostics of the world's most vulnerable population, neonates.

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PLATFORM

S09

Cocaine Hair Today, Gone Tomorrow: Unveiling the Impact of Cosmetic Treatments on External Contamination

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Abstract

Introduction: Interpreting drug testing results becomes a hair-raising challenge when external contamination comes into play. While previous studies have explored how cosmetic treatments affect drug removal from within the hair shaft, little is known about their impact on external contamination. This knowledge gap underscores the need for further investigation to comprehend how cosmetic treatments influence external hair contamination and enhance the accuracy and reliability of drug testing outcomes.

Objectives: This study aimed to investigate the influence of cosmetic treatments including dry shampoo, gel, henna, and bleach treatments, as well as a mixture of those, on the external hair contamination of cocaine (COC) and benzoylecgonine (BZE). Additionally, it was sought to determine whether these treatments mitigate or reduce contamination in hair samples of diverse shapes and colors.

Methods: Negative hair samples (n=16) were collected from a cohort of volunteers, which included various hair colors (black, brown, blond, and red) and textures (straight, wavy, curly, and kinky). The cosmetic treatments applied consisted of one of the following: dry shampoo; gel; henna or bleach. Split into three distinct groups, the hair samples underwent different treatment protocols: Group A experienced a cosmetic treatment before in-vitro contamination with COC and BZE; Group B was first in-vitro contaminated with COC and BZE prior to the referred cosmetic treatments; and Group C solely underwent contamination (control group). The process of in-vitro contamination involved immersing the samples in a 1 ug/mL COC/BZE solution in water for 24h, followed by overnight drying. All samples were washed prior to analysis with 10mL water, 10mL methanol and 10mL dichloromethane (2 min vortex each time) and both the hairs and their respective last wash were analyzed by a fully validated LC-MS/MS method.

Results

- For COC: Pre-Contaminated Hair (Group B): Bleach showed the most efficient removal of COC, resulting in an average concentration decrease of 90% compared to the contamination-only group (Group C). It was followed by dry shampoo (-70%), henna (-63%), and gel (-36%). Post-Contaminated Hair (Group A): Henna demonstrated the highest effectiveness in preventing subsequent external contamination, with an average concentration decrease of 90% compared to Group C. The subsequent rankings for prevention were bleach (-74%), dry shampoo (-72%), and gel (-51%).

- For BZE: The impact of cosmetic treatments on BZE contamination seemed to be less extensive compared to COC. Group B: The most significant reduction in BZE concentration occurred with bleach, showing a decrease of 76%. Gel, dry shampoo, and henna resulted in decreases of 53%, 51%, and 47%, respectively. Group A: Dry shampoo exhibited the highest reduction in BZE average concentration at -61%, followed by henna (-29%), bleach (-14%), and gel (-9%). All the above concentration declines were deemed to be statistically significant, determined by Wilcoxon and one-way ANOVA tests (p-values < 0.05).

Discussion: Previous studies have shown that henna might be responsible for creating an external protective layer on the hair shaft, hence explaining a decrease on cocaine contamination as seen on group A's COC hairs. Additionally, bleach is known to be a harsh type of cosmetic procedure and decrease drug concentrations in the hair shaft, that can be seen on group B's COC hairs. As for BZE, major decreases were not found. This might be due to the hydrolysis of COC to BZE, resulting in small losses of the metabolite. The findings do suggest that cosmetic treatments may have an important impact on external contamination of COC/BZE by removing existing drugs and potentially preventing further contamination.

PLATFORM

S10

Prevalence of Substance Use in US Court-ordered Mandatory Drug Testing of Oral Fluid and Hair

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Abstract

Introduction: Substance use disorders (SUD) in the United States are wreaking havoc on the nation, as evidenced by the increase in law enforcement drug seizures and overdose deaths. A major issue contributing to overdoses is suppliers mixing multiple drugs with deadly consequences.

Approximately 65% of the U.S. prison population has an active SUD (NIDA, 2020). Court-ordered mandatory drug testing (COMDT) is frequently conducted in correctional settings to determine abstinence compliance or usage history (e.g., probation, parole, custody). Furthermore, prevalence data using oral fluid and hair testing are sparse.

Objective: This observational study seeks to provide timely, evidence-based intelligence on growing rates of drug use and patterns of substance use in individuals enrolled in COMDT programs.

Methods: This 5-year retrospective analysis of COMDT data detects substances in oral fluid and hair collected in nationally represented COMDT programs. 959,237 oral fluid and 65,645 hair specimens were analyzed for misused substances representing a random, national COMDT sampling from 2015 to 2019. All specimens were screened by immunoassay and confirmatory testing was performed on a subset of oral fluid and all hair positive specimens by LC-MS/MS or -HRMS. Direct statistical inferences were determined for the larger population of all COMDT specimens. Similarly, the prevalence of positive drug tests among the analysis pool were estimated. Multiple specimens from the same individual, when available, were assessed using longitudinal analysis with a pseudo-design structure.

Results: COMDT drug prevalence data can be compared to other prevalence data such as U.S. workforce testing. Amphetamines, opioids, cocaine, benzodiazepines, cannabinoids, and phencyclidine were detected. The screening positivity rate in oral fluid was 34.0% with 7.8% total confirmed, whereas the hair overall positivity rate was 56.0%. This is up to 5 times higher than oral fluid and hair in a U.S. workforce population during a similar time period (10.2% and 10.9%, respectively; Quest Diagnostics, 2018). Representative drug combination positivity rates were also determined.

Discussion: This is the first large-scale, recent drug prevalence study in a US COMDT population. Understanding long-term patterns of use and confirming compliance and periods of abstinence are important observations for COMDT populations. The use of hair and oral fluid over urine testing offers longer detection, less invasive specimen collection, and more difficulty for specimen adulteration or substitution. Detection of drugs in hair and oral fluid can also provide more focused and accurate data among special population surveillance. This study indicates that SUDs in COMDT populations (i.e., special population) may be more prevalent than workplace drug testing populations (i.e., general population). These results can serve as an early warning to help legal systems and public safety programs support treatment for SUDs.

S11

Prevalence of foam cones in postmortem cases and the relationship with opioid-related deaths

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Abstract

Introduction: Opioids act partially as central nervous system depressants that can cause respiratory depression leading to death. This respiratory depression can cause excess fluid to build up in the lungs producing foam or froth in the airway that can exude from the mouth and nose as a foam cone. Scene investigators, pathologists, and toxicologists are instructed to look for the presence of foam/foam cone around the nose and oral cavity as an indication of a possible opioid-related death. However, foam/foam cones are not specific to opioid-related deaths and their presence could be related to the use of other central nervous system depressants or health conditions. Conversely, the absence of foam/foam cone does not exclude the possibility of an opioid-related death.

Objectives: Evaluate the scene observation of foam/foam cones in opioid and non-opioid related cases to determine utility of this observation as an indicator of an opioid-related death.

Methods: Cases from 2012 to 2023 were queried for scene investigator notes of foam or foam cone and causes of death. Data were separated into three categories: opioid-related cause of death, non-opioid toxicology-related cause of death, and non-toxicology-related cause of death (e.g., cardiovascular disease, drowning, etc.). Additionally, opioid-related cause of death cases without the presence of foam or foam cone in the scene investigator notes were collected to evaluate the overall prevalence in opioid-related cases. These were evaluated for trends and observations.

Results: There were 966 cases identified that had mention of foam/foam cone in the scene investigator notes. Of those 966 cases, 92 (9.5%) cases were non-toxicology-related cause of death, 170 (17.5%) cases were non-opioid toxicology-related cause of death, and 704 (72.8%) cases were opioid-related cause of death. Of the 170 non-opioid toxicology-related cause of death cases, 72 (42.3%) had one or more central nervous system depressants listed in the cause of death. The 704 opioid-related cause of death cases with foam/foam cone was out of 4,374 opioid-related cause of death cases in the same time frame, representing only 16.0% of all opioid-related cause of death cases.

Conclusion/Discussion: While the presence of foam/foam cone at the scene investigation is not specific to an opioid-related death, the presence is overwhelmingly associated with opioid-related deaths. However, it should be noted that overall, a foam/foam cone presence in an opioid-related death case is not commonly observed as it is seen in less than 1/5th of the cases. Therefore, the absence of a foam/foam cone does not preclude the possibility of an opioid-related death and should not be relied upon solely for initial evaluation of type of case or whether toxicology testing is needed. Limitations of these results are that the scene investigator had to note the presence of foam or foam cone in their report. Scene photos were not reviewed to confirm or identify cases with foam/foam cone. Autopsy findings of pulmonary edema or foam in the airway were not included for these cases. Both limitations could lead to an underrepresentation of the presence of foam/foam cone in all case types; however, using the presence of foam/foam cone at the scene to suggest the death is opioid-related can be useful for requesting toxicology testing.

PLATFORM

Emergence of Fentanyl-Related Deaths in Travis County, Texas, and Surrounding Areas

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Abstract

Introduction: Fentanyl and its analogues continue to be at the forefront of the opioid epidemic in the United States (US). In a drug market previously controlled by heroin, fentanyl has become the dominant opioid due to its ease of synthesis and desirable pharmacological effects. Even with the US attempt to control illicit fentanyl entering the country, fentanyl-related toxicities and fatalities continue to increase. In the South, fentanyl-related drug reports increased significantly beginning in 2019, however, there is a relative lack of information characterizing fentanyl-related fatalities specifically in this US region. This study aimed to examine fentanyl-related fatalities in Travis County, Texas (TX) and surrounding areas, housing the Austin-Round Rock metropolitan area with a projected population of 1,305,154 (2022). Austin sits as the fourth largest city in TX, the eleventh most populous city in the US and was ranked the second fastest-growing city in the US in 2022.

Objective: Given the increased prevalence of fentanyl in the Southern region of the US coupled with the influx of people moving to Travis County in recent years, the postmortem fentanyl-related drug toxicities from 2020 to 2022 were examined. A retrospective analysis was conducted to characterize these deaths in Travis County and surrounding areas to establish trends observed in demographic and toxicological data over this three-year period, to gain a better understanding of the fentanyl epidemic in this area.

Method: Postmortem cases submitted to the Travis County Medical Examiner for toxicology testing between January 1, 2020 and December 31, 2022, from Travis County, TX, and 45 surrounding counties, were queried to identify the total number fentanyl-related drug toxicities reported during this timeframe. Demographic (sex, race, age) and toxicological (fentanyl concentrations, norfentanyl concentrations, and additional/concurrently reported drugs) information was gathered for all fentanyl-related fatalities and evaluated annually and in totality. Additional information obtained through the medicolegal death investigation, such as scene findings and observations, was also evaluated for potential trends over time.

Results: Fentanyl contributed to 2.6% (36/2365) and 12.2% (299/2441) of deaths submitted for toxicology in 2020 and 2022, respectively, representing a 375% increase in fentanyl-related deaths over this three-year period (517/7087). Fentanyl-related fatalities primarily occurred in White males in their mid-30's. Fentanyl and norfentanyl concentrations ranged from 0.58-320 and 0.53-140 ng/mL with mean (median) concentrations of 17.2 ± 25.0 (11.0) and 5.6 ± 10.9 (2.9) ng/mL, respectively. Significant increases in fentanyl concentrations were observed in 2021 and 2022 compared to 2020. Polydrug use was also present in 88% of cases, with methamphetamine (or other amphetamines) (25%), benzodiazepines (21%), and cocaine and/or metabolites (17%) representing the most frequently concurrently identified substances. Although co-positivity rates of various drugs and drug classes widely varied over time, methamphetamine (and/or other amphetamines) was found in 56% of fentanyl-related deaths in Q4 of 2022. Scene investigations reported illicit powder(s) (n=141) and/or illicit pill(s) (n=154) in 48% (n=247) of fentanyl-related deaths. Illicit oxycodone (44%, n=67) and illicit 'Xanax' (38%, n=59) pills were frequently reported on scene; however, toxicology only identified oxycodone and alprazolam in 2 and 24 of these cases, respectively.

Discussion: Detailed analyses of toxicological information from fentanyl-related fatalities highlighted the onset and extent of the fentanyl epidemic in Travis County and surrounding areas over the past three years. Although drastic increases in fentanyl-related deaths were observed from 2020-2022, overall positivity rates remained relatively low compared to other regions of the United States. The results of this study helped provide a better understanding of the fentanyl epidemic in this region creating an opportunity to promote increased awareness, shift focus to harm reduction, and aid in minimizing public health risks.

Bitter Brew: A Wake-Up Call on Caffeine-Induced Fatalities

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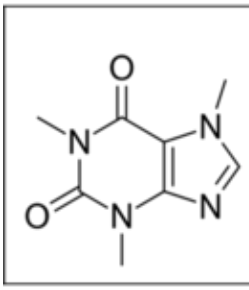
Abstract

Introduction: Caffeine is a naturally occurring drug and one of the world's most widely consumed psychoactive substances. The bitter methylxanthine alkaloid is found in dozens of plant species, including *Coffea arabica*, *Camellia sinensis*, and *Theobroma cacao*, from which we obtain coffee, tea, and chocolate, respectively. The immense popularity of caffeine stems from its stimulant properties, which increases alertness, elevates mood, and wards off fatigue. In traditional preparations, caffeine content can range from approximately 20-200 mg per serving and is generally well-tolerated by the consumer. Complications can arise, however, with the addition of synthetic or extracted caffeine to products like energy drinks and supplements. These products frequently have caffeine content exceeding 200 mg, with pure powdered caffeine reaching 3200 mg in a single teaspoon. In addition to everyday use, caffeine is identified as one of the most common adulterants in illicit drugs in varying and unknown amounts. Symptomology of caffeine toxicity includes classic central nervous system stimulation side effects such as agitation, insomnia, gastrointestinal distress, tachycardia, and seizures. In extreme cases, death could occur.

Objectives: Caffeine is a common finding in postmortem toxicology casework and significant findings could be dismissed when considering other detected drugs or simply overlooked entirely. This presentation will discuss the results of postmortem caffeine concentrations and use case studies where caffeine was the cause of death or played a significant role.

Methods: Routine postmortem testing via liquid chromatography time-of-flight mass spectrometry at NMS reports caffeine qualitatively as "presumptively positive." Quantitative confirmations come from either direct testing requested by the client at the time of specimen submission or recommended by an NMS toxicologist during case review. Confirmations are achieved by triple quadrupole liquid chromatography tandem mass spectrometry using a fully validated method with an analytical range of 0.10 to 20 mg/L. For this study, quantitative caffeine data from postmortem blood samples over an approximately 4.5-year period (January 2019 - May 2023) were collected and grouped into three categories: potentially therapeutic, toxic, and lethal.

Results: Cases reporting quantitative caffeine (n = 295) exhibited a wide range of concentrations, from 0.1 – 440 mg/L. Of these positive cases, 229 (78%) were in a therapeutic range of 0.1 to 25 µg/mL, 26 (9%) were in a proposed "toxic" range of 26 to 75 mg/L, and 40 (13%) were potentially lethal at concentrations greater than 75 mg/L.



Caffeine Bin	Range (mg/L)	n	Mean (mg/L)	Median (mg/L)
"Therapeutic"	0.1 – 25	229	5.92	2.70
"Toxic"	25 – 75	26	49.1	49.5
"Lethal"	>75	40	177	160
TOTAL	**0.1 – 440**	**295**	**32.9**	**4.80**

Discussion: The detection of caffeine is extremely common in postmortem toxicology, and in the vast majority of cases, it is at a therapeutic concentration that does not relate to the cause and manner of death. Still, caffeine-related fatalities exist. The availability of caffeine-containing products at high concentrations (e.g. pills and powders) lends itself as a source for accidental and intentional overdoses as well as a cause of detrimental side effects commonly associated with stimulants. Laboratories and toxicologists should be aware that caffeine could be a "blind spot" for them, with significant caffeine concentrations possibly overlooked, and develop means to catch cases that appear to be elevated.

PLATFORM

Household Harms: The Investigation of Deaths Caused by Household and Commercial Products

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Abstract

Introduction: In 2021, the American Association of Poison Control Centers reported more than 200,000 human exposures to household chemical products across the United States. Many reported cases were a result of accidental or intentional exposure at a person's primary residence, and the largest affected population was children aged five years and younger. Forms of exposure largely included ingestion or inhalation due to improper use, storage, or application. Although forensic toxicology laboratories largely deal with illicit substances, over-the-counter medications, and prescription medications, everyday household items are unfortunately often overlooked. These compounds can be readily purchased at local supermarkets and/or home improvement stores. Key risks of industrial and commercial product exposure include kidney damage, central nervous depression, and gastrointestinal damage, with other side effects ranging from headaches, dizziness, slurred speech, altered mental status, nausea, vomiting, respiratory depression, loss of consciousness, and/or death. Unfortunately, household items are also easily abused for their euphoric effects, which can increase the likelihood of fatal outcomes.

Objectives: The objective of this presentation is to discuss the need for alternative toxicological testing methods for the detection of household chemical products, as well as the necessity for collaboration between toxicologists, pathologists, and investigators when faced with cases involving accidental or intentional exposure to these types of products.

Methods: Three representative cases were chosen to illustrate the need for adaptable testing methods and an expanded scope of testing in forensic toxicology laboratories. For this study, household products include those found within the home (e.g., cleaning products), and commercial chemicals (e.g., paint thinner, antifreeze, etc.) that are not intended for human consumption. Case 1 is a 77-year-old white male who ingested an unknown quantity of paint thinner. Case 2 involves a 56-year-old white male found decomposed with a known history of anti-freeze consumption. Case 3 is a 16-year-old white female found deceased with a can of nail enamel dryer. These cases and their corresponding evidence underwent routine toxicological analysis, and then further testing for volatiles by solid-phase microextraction procedure (SPME) and gas chromatography-mass spectrometry analysis (GC-MS) or ethylene glycol by protein precipitation and gas chromatography-tandem mass spectrometry analysis (GC-MS/MS).

Results: Case 1 was analyzed for volatile compounds; methanol, methylene chloride, octane, and toluene were detected in antemortem blood, postmortem blood, and scene evidence (paint thinner). In addition, octane and toluene were detected in the gastric contents for this case, which confirms the product was ingested. Ethylene glycol was detected in Case 2 at 2,051 mg/Kg and 9,456 mg/L in liver and urine, respectively. Finally, the lung blood and scene evidence (nail enamel dryer) from Case 3 were analyzed; isobutane was detected in both samples in addition to other volatile hydrocarbons. In all three cases, toxicological analysis was instrumental in helping pathologists to accurately determine cause and manner of death.

Conclusion/Discussion: With the ease of availability of commercial and industrial products, it is no surprise that they are a common cause of accidental or intentional poisonings and/or fatalities. As a forensic toxicologist, special care must be taken when analyzing these cases, as they fall outside the realm of routine toxicological analysis. When possible, every effort must be made to obtain evidence from the scene so that it can be tested in conjunction with biological samples. To effectively determine cause and manner of death, toxicologists, pathologists, and investigators must openly communicate, evaluate the case in its entirety in terms of eyewitness accounts, terminal event circumstances, and scene evaluation, and adapt the toxicological testing accordingly.

S15

The Importance of Toxicological Findings in a Mass Casualty Event

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Abstract

Introduction: This presentation will highlight the importance of toxicology testing in a mass casualty event. In addition the toxicology results in whole blood will reflect concentrations that are most likely from therapeutic drug use. The mass casualty incident was a commercial diving boat fire with 34 victims. The investigators were faced with monumental pressure to recover bodies and conduct toxicology testing as soon as possible. Theories of the cause of the fire included a terrorist attack and a boat filled with intoxicated passengers.

Objectives: The objective of this presentation is to discuss the type of testing required, the order in which it was performed and the expedition of the results to the investigators. The attendee will learn the process of how to triage and test problem specimens from a high profile mass casualty event. The attendee will appreciate the importance of toxicology results to the investigation and the ultimate finding of the possible cause of the event. Attendees will also gain knowledge from drug concentrations in whole blood during therapeutic drug use.

Methods: The blood samples and one spleen sample were recovered from intact bodies, burned bodies and submerged bodies recovered at sea 2-3 days following the incident. The samples were screened by color screen for cyanide, micro-diffusion for carbon monoxide with confirmation of positive findings by the Avoximeter 4000 (AVOX 4000) when possible, headspace gas chromatography with flame ionization detection (GC/FID) for volatiles including ethanol, methanol, isopropanol and acetone, blood samples were screened by enzyme linked immunosorbent assay (ELISA) to include sympathomimetics, barbiturates, benzodiazepines, buprenorphine, carisoprodol, cannabinoids, cocaine metabolite, fentanyl, methadone, tramadol, zolpidem, phencyclidine, and opiates to include oxycodone and oxymorphone. All samples were screened by liquid chromatography-mass spectrometry-mass spectrometry (LC/MS/MS) in both multiple reaction monitoring mode (MRM) and full scan mode with confirmation of positive findings by gas chromatography-mass spectrometry (GC/MS) or LC/MS/MS. When needed analytes were extracted by either liquid:liquid or solid phase extraction.

Results: All victim samples were positive for carbon monoxide with a range of 39% to greater than 75% carbon monoxide saturation. Two unsuitable blood samples and the spleen were tested for carbon monoxide using the micro-diffusion method only. The table below shows the drugs detected, the number of occurrences and, the concentration or concentration range if more than one occurrence. There were 11 victims that were none detected for drugs.

Drug Detected	Number of Occurrences	Concentration or Range
Scopolamine	2	Unconfirmed finding
Meclizine	9	9.0 to 158 ng/mL
Diphenhydramine	4	5.2 to 81 ng/mL
Ephedrine/PSE	3	Qualitative confirm
Nortriptyline	1	88 ng/mL
Codeine	1	38 ng/mL
Trazone/mCPP	1	158/16 ng/mL
Bupropion	2	49 to 101 ng/mL
OH-Bupropion	2	136 to 490 ng/mL
Ondansetron	1	Unconfirmed finding
Cetirizine	1	45 ng/mL
Gabapentin	1	5310 ng/mL
Ethanol	9	0.01 to 0.05%
Cyanide	17	ND to < 2 mcg/mL

Discussion: The toxicology findings included the presence of carbon monoxide in every victim which does not support the cause of the fire being from a bombing or sudden explosion. There were no illicit drugs detected. Most drugs were prescription drugs or over the counter drugs including those used to prevent motion sickness. The concentrations appear to be in the therapeutic range. The findings of ethanol were in the presence of beta-phenethylamine suggesting the ethanol may have been from decomposition. There was no toxicological evidence that the passengers were abusing drugs or alcohol. In fact there was evidence that several of the passengers did try to escape the boat prior to being overcome with carbon monoxide.

Investigation of postmortem formation of ethanol using ethyl sulphate and 1-propanol

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Abstract

Introduction: Analysis of ethanol and other alcohols is routinely performed as part of the post mortem investigation. However, the detection of ethanol does not necessarily reflect ingestion since it can be formed during the decomposition process. During this process, in addition to ethanol, higher alcohols, such as 1-propanol and 1-butanol can also be produced. An approach to diagnose post mortem formation of ethanol is therefore to analyse for these markers. If markers are present, analysis of the direct ethanol metabolite, ethyl sulphate (EtS) is performed. A negative result for EtS excludes ingestion of ethanol. Ethyl glucuronide (EtG) was also analyzed but is considered a less reliable marker.

Objectives: The objectives of this study were to determine the occurrence of post-mortem formation of ethanol in autopsy cases and to investigate the relationship between the 1-propanol and ethanol concentrations in EtS negative cases.

Methods: Ethanol, 1-propanol, and 1-butanol were analyzed by head-space gas chromatography mass spectrometry on an Agilent Intuvo 9000 GC with a 5977B MS detector. Ethyl sulphate was analyzed using supercritical fluid chromatography on an Agilent Infinity II SFC with an Ultivo triple quadrupole MS detector. All consecutive samples between October 2022 and March 2023 where ethanol was positive in femoral blood and 1-propanol was detected were analyzed for EtS in femoral blood.

Results and Discussion: A total of 735 cases were positive (> 0.1 mg/g) for ethanol in femoral blood. Of those, 163 (22%) also presented with 1-propanol above the method cut-off 0.01mg/g. 1-butanol was also found above the 0.01 mg/g cut-off in 48 cases. The subsequent analysis of EtS in femoral blood was possible in 147 cases and in 90 cases (61%) EtS was negative (<0.01 µg/g) suggesting that all ethanol was formed postmortem. In two EtS negative cases EtG was above the 0.1µg/g cut-off. A summary of results from the 147 cases is presented in Table 1. There was only a weak relationship (r2=0.19) between the concentrations of 1-propanol and ethanol in EtS negative cases showing that only a small part of the variation in post mortem formation of ethanol could be explained by the simultaneous formation of 1-propanol. In five cases, high concentrations (1.5-3.1 mg/g) were found when EtS was negative confirming that considerable amounts of ethanol can be formed post mortem under certain circumstances. In those five cases the 1-propanol concentrations were between 0.03-0.15 mg/g.The ratio between the urine alcohol concentration (UAC) and blood alcohol concentration (BAC) is often used to confirm ethanol ingestion using a UAC/BAC>1.25 which is an expected ratio when ethanol has distributed in the body. However, in 30% of EtS negative cases the ratio was above 1.25, falsely pointing towards ingestion. In 1-propanol negative cases that had both blood and urine samples (N=340) 63% of the cases had a UAC/BAC>1.25 and 89% a ratio above 1.0. An occurrence of 22% cases is similar to previous published studies indicating that 0.01mg/g of 1-propanol is a good threshold for identifying post mortem formation.

Conclusions: We conclude that there was a weak positive correlation between 1-propanol and ethanol in cases where all ethanol had been produced post mortem. Post mortem ethanol could reach up to 3 mg/g and the use of UAC/BAC ratios was not sufficient for interpretation. Analysis of vitreous may have helped in interpretation.

Table 1.	EtS negative	cases (N=90)	EtS positive	cases (N=57
	Median	Range	Median	Range
B-Ethanol (mg/g)	0.38	0.12-3.12	0.89	0.12-3.90
B-1-propanol (mg/g)	0.026	0.010-0.213	0.027	0.011-0.118
B-1-butanol (mg/g)	0.024	0.011-0.666	0.02	0.011-0.331
B-EtS (µg/g)	-	-	0.41	0.01-3.32
B-EtG (µg/g)	-	0.12-0.14	0.44	0-8.86
UAC/BAC	1.0	0.19-2.1	1.2	0.45-2.6

Adverse events and mitragynine and 7-hydroxy-mitragynine plasma pharmacokinetics after controlled oral kratom leaf administration to healthy human participants

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Abstract

Introduction: The KAPTURE study is a randomized, double-blind, placebo-controlled, dose-escalation study to evaluate safety, tolerability, and single and 15-day repeated encapsulated oral dried kratom powder pharmacokinetics in healthy participants conducted by Johnson Foods and approved by Health Canada. National and international surveys show that kratom consumers self-report using kratom to self-manage opioid and other substance use disorders, to support energy and wellness, and for chronic pain relief. A minority consume much larger kratom doses, may develop dependence and may experience toxic effects. Kratom alkaloid content and concentrations vary widely and there is debate on the safety of kratom doses. Tremendous growth in kratom research, primarily funded by NIDA, highlights mitragynine's unique mechanisms of action due to partial agonism at the μ-opioid receptor without recruiting the beta-arrestin pathway responsible for respiratory depression and constipation; thus, NIDA's interest in investigating kratom as a harm reduction tool for opioid use disorder.

Objectives: Our aim is to provide extensive adverse event (AE) and pharmacokinetic data from KAPTURE, the largest controlled kratom leaf study, to improve interpretation of mitragynine and its active metabolite, 7-hydroxy-mitragynine (7-OH-MTG) concentrations. As there is no consensus on toxic concentrations, these single and steady-state 500 to 2000 mg kratom powder doses and AE add to the scientific database for interpreting mitragynine concentrations.

Methods: A placebo-controlled, ascending single and 15 consecutive daily 6.65, 13.3, 26.6 and 53.2 mg mitragynine dose study was conducted in 114 healthy individuals (48 receiving active and 66 placebo kratom) during 31 in-person visits with AE monitoring, comprehensive metabolic and hematology panel testing, and plasma sampling up to 10 and 23 days after single and multiple doses, respectively.

Results: There were no serious adverse events or deaths. There was no sustained or meaningful improvement or deterioration in alertness (cognitive task) after active or placebo mitragynine following normal or deprived sleep. The number of suspected to be related AE increased from 7 to 229 with increasing dose. At the highest single 53.2 mg mitragynine dose (n=13), dizziness (38.5%), nausea (30.8%), somnolence (23.1%), and headache, feeling drunk, feeling of relaxation, feeling hot and vomiting (all in 15.4% or 2 participants) occurred. After 15 days of the highest dose (n=12), feeling hot (41.7%), headache and feeling drunk (33.3%), alanine transaminase (ALT) increase, nausea, somnolence, and dizziness all at 25.0%, and constipation, feeling abnormal, aspartate transaminase (AST) increase, and decreased appetite in 16.7% or 2 participants were noted. Three participants were terminated early at the highest dose due to AE, 1 voluntarily due to multiple "suspected to be related" AE, and 2 from elevated ALT and elevated AST during the multiple-dose phase. All participants recovered from all AE at the end of the study. After the highest single oral dose of 53.2 mg mitragynine (2000 mg kratom leaf powder), median (range) mitragynine Cmax was 130 (34.2-204) ng/mL, Tmax 1.3 (0.75-2.0) h, and T1/2 42.9 (8.3-84.5) h and the same parameters for 7-OH-MTG were 21.7 (12.5-38.6) ng/mL, 1.7 (1.0-2.3) h, and 4.0 (1.7-11.4) h, respectively. After 15 daily doses of 53.2 mg mitragynine, median mitragynine Cmax was 155 (64.3-215) ng/mL, Tmax 1.7 (1.0-3.0) h, and steady-state T1/2 was 48.6 (31.3-83.8) and the same parameters for 7-OH-MTG were 20.9 (13.3-31.7) ng/mL, 2.0 (1.3-3.5) h and 9.1 (2.2-71.6) h, respectively. The 7-OH-MTG/mitragynine ratio was higher during single-dosing than during multiple-dosing, and at the lowest rather than highest doses, with a maximum ratio of 0.5 following 6.65 mg single dose mitragynine.

Discussion: These controlled kratom leaf human administration data provide critically needed AE and pharmacokinetic data for interpreting potentially therapeutic and toxic mitragynine and 7-OH-MTG concentrations.

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Assessing the Impact of Anabolic-Androgenic Steroids on Drug-Drug Interactions: In-Vitro Evaluation of Metabolic Clearance of Common Illicit and Medicinal Compounds

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Abstract

Introduction: Drug clearance plays a vital role in determining the efficacy and safety of a drug, and can be influenced by drug-drug interactions (DDIs). DDIs occur when multiple drugs are present simultaneously in the body and the pharmacology of one or more is altered by another. Metabolic DDI can occur through various mechanisms, including competition for, or inhibition of, metabolic enzymes or transporters responsible for drug clearance and distribution. This can result in slowed drug clearance or altered metabolite formation.

Illicit use of anabolic-androgenic steroids (AASs) has increased in recent years, including polydrug use with cocaine and MDMA. AAS misuse has been linked with greater psychopharmacological treatment, while recent research has also detected the presence of AASs alongside synthetic cannabinoid receptors agonists. Currently there is a lack of information regarding the impact of DDIs on AASs and analyte clearance.

Objectives: To evaluate the impact metandienone, nandrolone and testosterone have on the metabolic clearance of ADB-FUBIATA, CH-PIATA, cocaine, MDMA, and quetiapine via in-vitro human liver microsome (HLM) incubations.

Methods: Intrinsic clearance of test compounds was measured in HLM incubations both with and without AASs. HLM (final concentration 0.5 mg/mL microsomal protein) solutions were prepared in potassium phosphate buffer (50 mM, pH 7.4) and, where appropriate, pre-incubated with each AAS (each at 10 µM, consistant with levels of exogenous use) as potential inhibitors (5 min, 37 °C, shaking at 100 rpm). Test compounds and verapamil (positive control) (each at 0.5 µM) were added to incubations, and then 50 µL of NADPH (8 mg/mL) was used to initiate the reaction (final incubation volume 500 µL). Samples were incubated (37 °C, shaking at 100 rpm) and 50 µL samples were transferred to 200 µL acetonitrile (containing donepezil internal standard (10 ng/mL)) to stop the reaction at 0, 3, 6, 9, 15, 30, 45 and 60 min(s). Following incubation, 80 µL dH_2O was added to each sample before centrifugation (3750 rpm, 20 °C, 10 min). UPLC-MS/MS analysis was achieved by gradient elution using a Waters Acquity UPLC (column: Acquity BEH C18 (2.1 x 50mm, 1.7µm) coupled with a Waters Xevo TQ-S MS. Peak area ratio was plotted against incubation time to determine half-lives ($t_{1/2}$) and intrinsic clearance rates (CL_{int}) of parent analytes with and without the presence of AAS as potential inhibitors.

Results: Clearance of cocaine, MDMA and quetiapine were unaffected showing that their phase I metabolism by HLM is not significantly affected by the presence of these AASs. ADB-FUBIATA was also unaffected, however, we can report here for the first time the experimental HLM clearance of this compound in isolation ($t_{1/2}$ = 9.82 ± 1.38 min). The intrinsic clearance of CH-PIATA was $t_{1/2}$ = 4.04 ± 0.47 min. CH-PIATA was found to be cleared at a slower rate in the presence of AASs, with CH-PIATA clearance as follows; with metandienone, $t_{1/2}$ = 12.42 ± 3.84 mins; with nandrolone, $t_{1/2}$ = 8.71 ± 2.56 mins; with testosterone, $t_{1/2}$ = 9.09 ± 2.48 mins.

Discussion: The co-incubation of metandienone with CH-PIATA resulted in, on average, a 3-fold reduction in CH-PIATA intrinsic clearance, while nandrolone and testosterone caused an average 2-fold reduction compared to CH-PIATA alone. This preliminary data suggests that high-dose AAS use may significantly affect CH-PIATA clearance in vivo.

PLATFORM

High body mass index is prevalent in people with opioid use disorder in Northern England and increases risk of death

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Abstract

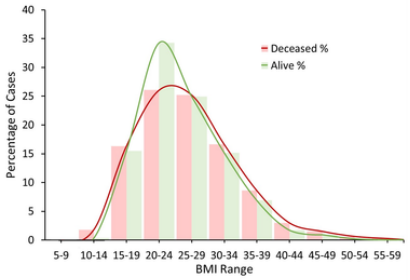
Introduction: Opioids are used clinically for their analgesic and sedative properties but are also misused in non-clinical scenarios for their euphoric effects. Tolerance to opioids rapidly develops, meaning a user must administer increasing amounts of the same drug to achieve the same effects. At high doses the sedative effects of opioids can cause respiratory depression and slowed heart rate, which in severe cases can lead to death.

People who are obese are at higher risk of suffering from other health conditions including obstructive sleep apnoea and cardiac disease due to increased levels of fat deposits – obstructive sleep apnoea when these fat deposits are in the upper respiratory tract where they narrow the airway and restrict breathing, and cardiac disease, such as atherosclerosis and increased blood pressure, when these fat deposits are in the vasculature and narrow blood vessels. Such co-morbidities may increase risk of death from opioid use.

Objectives: This research examined a population of people living in the North of England with opioid use disorder to understand if those who are overweight – here defined by body mass index (BMI) – are at increased risk of death in comparison to people of a healthy weight (BMI 18.5-24.9). BMI was chosen as the metric to define 'healthy weight' as the height and weight measurements with which BMI is calculated are routinely recorded by Drug & Alcohol Services in the UK, whereas percentage body fat is not.

Methods: Data were extracted from Drug & Alcohol Treatment Services at two locations in Northern England (Derbyshire and Teesside) between May 2022 and June 2023 regarding people who were registered as receiving opioid agonist therapy (methadone or buprenorphine). A binomial linear regression model was used to calculate the adjusted odds ratio and confidence intervals (CIs; at 95%), with Student's T test used to determine statistical significance using IBM SPSS Statistics software (version 27). The potential confounding factors of age, gender and deprivation decile of usual address were included in the regression model.

Results: A total of 1,917 cases were collected and comprised 1,580 living individuals and 337 people who had died a drug-related death. The mean BMI of both living individuals and decedents were above the threshold considered a healthy weight (≤24.9), with the mean BMI for individuals who had died (26.58±7.42) significantly higher than living individuals (25.63±6.35; p=0.015; Figure 1). Calculating an adjusted odds ratio considering age, gender and the deprivation decile of the decedents' usual addresses indicates a 2.4% increased risk of death for every additional BMI point (aOR 1.024; 95% CIs 1.006 – 1.042). There was no effect of age (aOR 0.994; 95% CIs 0.980 – 1.008) or gender (aOR 0.831; 95% CIs 0.634 – 1.089), with decedents living in more deprived areas (1 most deprived; 10 least deprived) at increased risk of death (aORs 0.871; 95% CIs 0.820 – 0.925).



Discussion: Heavy weight and obesity is prevalent in the studied population of people with opioid use disorder, with increasing weight representing a risk factor for death. This is in contravention to the 'heroin thin' rhetoric often associated with opioid use. People who use opioids and their healthcare treatment providers should be made aware of this risk and take steps for these people to achieve a healthy weight. Toxicologists, pathologists, and coroners should consider decedent BMI when interpreting post-mortem findings to ascertain cause of death.

Future work in this remit should consider additional potential confounding factors (e.g., polypharmacy, co-morbidities, smoking status) that were not possible to include in this analysis due to missing data.

S20

Challenges with Fentanyl renewed use interpretation.

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Abstract

Introduction: Fentanyl is a potent synthetic opioid used to treat severe pain. Fentanyl and norfentanyl, a metabolite and precursor of fentanyl, are detected in urine following fentanyl use. Although fentanyl is available by prescription, the illicit manufacture and use of fentanyl has significantly contributed to the current opioid epidemic in the United States.

In general, short-acting opioids are detected in urine for around 72 hours. However, extended elimination of fentanyl has been reported in persons with opioid use disorder (OUD).

Urine drug testing is one of the primary methods to detect use of illicit substances. Individuals in probation, recovery, employment, and healthcare settings rely on laboratory test results to identify whether new use of drugs occurred. Test results must be assessed closely as significant consequences occur as a result of suggested renewed use.

Objectives: The objective of this study was to review the anti-mortem fentanyl cases where reported drug cessation happened, but urine positivity continued for several weeks/months to understand the challenges with test result interpretation.

Methods: Donor data collected from urines confirmed positive for fentanyl and norfentanyl were used in this study. Six individual cases involving multiple urine test results submitted over a month or longer were reviewed. Individuals in the study self-reported cessation of use before or during the collection timeframe. Time between urine collections was variable, but frequency of collections was in the range of 1-3 collections per week. Fentanyl concentrations were normalized to 100 micrograms/deciliter creatinine levels to account for differences in urine dilution.

Urine samples were screened for fentanyl on a Beckman AU5840 analyzer using the Fentanyl Urine SEFRIA TM Drug Screening Kit (cutoff: 1 ng/mL). Creatinine concentrations were measured using the DRI TM Creatinine-DetectTM Specimen Validity Test on the same analyzer platform. Confirmation was performed by liquid chromatography tandem mass spectrometry (LC-MS/MS). The analytical range for fentanyl and norfentanyl in urine was 0.5 ng/mL to 500 ng/mL.

Results/Discussion: Specimens remained positive for norfentanyl (> 0.5 ng/mL) for at least one month in five out of the six cases. Since last admitted use, fentanyl concentrations ranged from < 0.5 ng/mL to 234.7 ng/mL while norfentanyl concentrations ranged from < 0.5 ng/mL to > 500 ng/mL over the course of 21 to 114 days. Additionally, fluctuations in the norfentanyl:creatinine ratio were observed that did not follow a continued decrease with time. Medication history was available for four cases and did not include fentanyl. In one case, the donor was pregnant within the collection timeframe.

In the scientific publication titled Protracted Renal Clearance of Fentanyl in Persons with Opioid Use Disorder, Huhn et al. concluded that fentanyl clearance may be considerably longer in individuals with OUD. In another case report, Delayed Norfentanyl Clearance During Pregnancy by Wanar et al., a pregnant woman with OUD was reported to have detectable norfentanyl in urine for 70 days following cessation.

Although these six cases involve self-reported cessation of fentanyl use, they support the limited published literature suggesting extended elimination of fentanyl/norfentanyl and highlight the need to exercise caution when concluding a positive drug test is due to renewed illicit fentanyl use.

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PLATFORM

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Survey of Forensic Laboratories Testing for Novel Psychoactive Substances (NPS)

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Abstract

Introduction: Since 2021, the SOFT NPS Committee has produced quarterly NPS Scope Recommendations to provide guidance to laboratories looking to develop or update their testing scope to include the timeliest and commonly identified NPS on the market. These scope recommendations are developed as a collaborative effort by members of the NPS Committee, spanning various regions of the United States, with additional perspectives and input from other national and international partners and reports.

Objectives: In early 2023, the SOFT NPS Committee sought to conduct a survey of forensic laboratories testing for NPS to determine both the effectiveness of our scope recommendations and also other pertinent information regarding their testing (e.g., instrumentation, NPS subclasses tested for, prevalence of specific NPS, etc.). The primary purpose of the survey was to solicit feedback about who uses the scope recommendations and how they are being used. The survey also allowed for suggestions on future improvements and developments.

Methods: The survey was developed in SurveyMonkey and facilitated online. The survey consisted of 29 total questions, including a mix of required and optional single answer, multiple choice, and open-ended response types. The estimated completion time was 10-15 minutes. The survey was distributed to the SOFT membership via email, as well as to the TIAFT membership and the CFSRE's NPS Discovery listserv. Generic questions included basic information about the respondent's laboratory, the type of work conducted (e.g., forensic toxicology, drug chemistry) and subdiscipline (e.g., postmortem, DUID), size of agency, years of experience testing for NPS, and others. Analytical questions included information regarding types of NPS tested for and instrumentation used for testing. There were nine questions regarding the effectiveness of the scope recommendations, including responses about frequency of development/distribution (e.g., quarterly, annually), utility of suggested cutoff concentrations, and manner in which the recommendations impact laboratory practice. The survey also included optional questions regarding NPS detections and prevalence, including a full list of all NPS previously listed in prior scope recommendations.

Results: Survey responses were exported to Excel and standardized prior to analysis. In total, there were 83 respondents that completed the survey, or a portion thereof. Respondents were primarily from the United States (77%), with responses from 14 additional countries. Respondents reported working for laboratories across 31 states, with the majority being public agencies (71%). Respondents reported conducting toxicology (84%) and drug chemistry (36%) testing with overlap, including work related to following fields: postmortem (65%), DUID (58%), DFC/DFSA (47%), clinical (25%), and drug material (43%). Respondents primarily (92%) worked for laboratories with less than 60 employees and caseloads were predominantly (83%) greater than 1,000 cases per year. Experience with NPS was approximately broken in thirds: less than 5 years (35%), 6-10 years (30%), and greater than 10 years (35%). LC-QQQ-MS (69%), GC-MS (65%), and LC-HRMS (42%) were the most commonly used methodologies reported for NPS testing. Respondents reported variance in NPS subclasses tested for: benzodiazepines (86%), opioids (84%), stimulants (76%), hallucinogens (60%), and cannabinoids (57%). Most respondents reported using the scope recommendations to expand scope of testing (64%) and to add new NPS to screening methods (54%). Most respondents (74%) said the scope recommendations were somewhat to highly useful, and most (64%) said quarterly regularity was appropriate (19% responded biannually, or two times per year). Finally, respondents reported that the SOFT NPS Scope Recommendations (66%) were their go-to resource for determining their scope of testing – the highest response selected.

Discussion: The SOFT NPS Committee successfully completed a survey of forensic laboratories regarding their NPS testing practices. Diversity in responses was noted, and both close- and open-ended responses provide the committee important insights moving forward.

Prevalence of Novel Psychoactive Substances in Routine Prescription Drug Monitoring Clinical Urine Specimens

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Abstract

Introduction: Novel psychoactive substances (NPS), also known as "legal highs", are designed to have similar effects to recreational drugs while circumventing legality issues. Use began to rise in 2010 and, as of 2022, more than 1,000 different NPS have been identified. While many forensic laboratories test for these analytes due to both investigative and post-mortem circumstances, clinical findings and clinical implications are not well studied.

Objectives: The objective of this surveillance study was to determine the frequency of NPS compounds in deidentified remnant clinical toxicology urine specimens from a large clinical drug testing laboratory.

Methods: Deidentified remnant specimens that had been submitted for clinical drug testing were analyzed over the course of 3 weeks in March 2023. A 3-point calibration curve was used to quantitate 85 NPS compounds in 6 NPS categories: fentanyl analogs, designer benzodiazepines, designer opioids, designer stimulants, synthetic cannabinoids, and other illicit compounds. Limits of detection ranged from 0.5 ng/mL to 5.0 ng/mL for all analytes. All NPS compounds were obtained from Cerilliant (Round Rock, TX), Cayman Chemical Company (Ann Arbor, MI), or Toronto Research Chemicals (North York, ON, Canada). Solvents were obtained from Fisher Scientific (Hampton, NH) or Millipore Sigma (St. Louis, MO). A dilute-and-shoot method was used to prepare urine specimens. An internal standard (IS) mix containing 14 deuterated IS compounds in methanol was added to the urine specimens and room temperature hydrolysis was performed using B-One, a synthetic β-glucuronidase enzyme (Kura Biotech, Los Angeles, CA). Specimens were then diluted with a mix of mobile phase A (10 mM ammonium formate and 0.1% formic acid in water) and mobile phase B (0.1% formic acid in 75:25 methanol/acetonitrile). Analytes were resolved using a Nexera XR LC-20AD LC (Shimadzu, Columbia, MD) with a 3.4-minute gradient on a Phenomenex Kinetex 50x3 mm F5 column coupled to a SCIEX 5500 QTRAP tandem mass spectrometer (Framingham, MA).

Results: In total, 1,784 specimens were analyzed. Of these, 127 (7.1%) tested positive for an NPS. Of the positive specimens, 90 (71% of positives, 5% overall) contained only one NPS class and 37 (29% of positives, 2.1% overall) contained two or more NPS classes. The class with the highest specimen positivity was designer benzodiazepines with 42 specimens (2.4% overall) containing at least one designer benzodiazepine. Overall, at least one compound from each of the 6 NPS classes was seen every week.

Analytes detected included fluorofentanyl, methoxyacetyl fentanyl, acetyl fentanyl, hydroxybromazolam, bromazolam, 8-aminoclonazolam, 4Cl-deschloroalprazolam, deschloroetizolam, etizolam, desethyl isotonitazene, 4-hydroxynitazene, eutylone, pentylone, dimethyl pentylone, cyclohexyl methylone, propyl butylone, MDMB-4en-PINACA COOH metabolite, 4-hydroxy xylazine, and xylazine.

Xylazine was the most commonly detected analyte, observed in 41 (2.3% overall) specimens. Of the specimens containing xylazine, 34 were seen with fentanyl and fentanyl analogs and 7 samples had no presence of fentanyl or fentanyl analogs. In these 7 specimens, xylazine was found mixed with at least one of the following compounds: hydroxybromazolam, bromazolam, hydroxynitazene, dimethyl pentylone, pentylone, and MDMB-4en-PINACA COOH metabolite. Dimethyl pentylone was the next most commonly seen analyte, observed in 36 (2.0%) specimens.

Discussion: In the 1784 randomly selected clinical toxicology specimens that were analyzed during the surveillance study, NPS were detected in 7.1% of specimens. Treatment decisions may be predicated upon NPS urinalysis results. This surveillance information can provide physicians insights into the necessity of NPS testing for higher-risk individuals in prescription drug monitoring programs; without NPS testing an individual may appear compliant with prescribed medications. NPS in clinical samples may also give insight into medicolegal matters and help identify when a drug has been obtained illicitly and what else may have been in the drug.

S23

Lipophilicity, plasma protein binding and intrinsic clearance of fentanyl analogues

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Abstract

Introduction: Fentanyl analogues have infiltrated the illicit drug market within the USA, causing grave concerns for public health and law enforcement agencies. These synthetic opioids, derived from fentanyl, have gained popularity among drug traffickers due to their high potency and potential for profit. The introduction of fentanyl and fentanyl analogues to the USA and Canadian illicit opiate supply is thought to play a significant role in the increase in synthetic opioid related deaths, which rose 18% in 2021. Despite widespread concerns surrounding their use, limited pharmacological data for these new analogues is available.

Objectives: This research aims to identify and investigate the lipophilicity, plasma protein binding (PPB), and intrinsic clearance of fentanyl and 14 fentanyl analogues (4-fluorobutyrfentanyl, 4-fluoroisobutyrfentanyl, acetylfentanyl, acrylfentanyl, butyrfentanyl, carfentanil, crotonylfentanyl, cyclopropylfentanyl, furanylfentanyl, methoxyacetylfentanyl, ocfentanil, *ortho*-fluorofentanyl, *para*-fluorofentanyl and valerylfentanyl).

Methods: LogD (pH 7.4) was determined using a HPLC-PDA-based chromatographic hydrophobicity index (CHI) method. A calibration mix of ten reference compounds was analysed at the start and end of each test run, with test compounds injected in triplicate. Average retention times of calibration compounds were used to calculate retention factors (k), which were then plotted against literature CHI values (CHI_0). Experimental CHI and CHI logD values were subsequently calculated.

PPB was determined by equilibrium dialysis. Pooled human plasma was centrifuged (3750 rpm, 10 min, 22 °C) and spiked with test compounds or positive control (nicardipine) (3 µg mL^{-1}). Equilibrated, spiked plasma was dialysed against isotonic phosphate buffer (pH 7.4) (5 h, 37 °C, 100 rpm). ACN containing donepezil internal standard (10 ng mL^{-1}) was added to each sample which were then centrifuged (3750 rpm, 10 min, 22 °C). The supernatant was diluted in dH_2O prior to UPLC-MS/MS analysis. The percentage of drug bound, and fraction unbound were calculated.

For intrinsic clearance incubations, test compounds and a positive control (verapamil) were incubated at 0.5 µM in HLM (0.5 mg microsomal protein mL^{-1} in 50 mM phosphate buffer, pH 7.4). NADPH in phosphate buffer (final concentration 0.8 mg mL^{-1}) was used to initiate the reaction (500 µL total incubation volume). Plates were incubated (37 °C, 100 rpm) and samples were collected from 0 to 60 min and quenched in ACN containing donepezil (10 ng mL^{-1}). Samples were diluted with dH_2O and centrifuged (3750 rpm, 10 min, 22 °C) prior to UPLC-MS/MS analysis. Rate constants (k, min^{-1}), half-lives ($t_{1/2}$, min), microsomal intrinsic clearance ($CL_{int\ micr}$, mL min^{-1} mg^{-1}) and intrinsic clearance (CL_{int}, mL min^{-1} kg^{-1}) rates were calculated. *In vivo* hepatic clearance (CL_H, mL min^{-1} kg^{-1}) was estimated according to the 'well-stirred' model.

Results: $LogD_{7.4}$ results ranged from 3.42 (methoxyacetylfentanyl) to 6.11 (valerylfentanyl). PPB ranged from 96.8% (valerylfentanyl) to 31.6% (acetylfentanyl). *In vitro* $t_{1/2}$ ranged from 7.5 (furanylfentanyl) to 53.0 mins (methoxyacetylfentanyl). This resulted in CL_{int} rates ranging from 250 (furanylfentanyl) to 35.4 (methoxyacetlfentanyl) mL min^{-1} kg^{-1}. CL_H was estimated to range from 16.84 (acetylfentanyl) to 2.64 (cyclopropylfentanyl) mL min^{-1} kg^{-1}.

Conclusion/ Discussion: The effect of various structural changes between fentanyl analogues on the tested properties were established. For example, increasing alkyl chain length (i.e. acetylfentanyl > fentanyl > butyrfentanyl > valerylfentanyl) resulted in increased lipophilicity, increased PPB, and an overall slower rate of predicted hepatic clearance. Fluorination (fentanyl vs *ortho*-fluorofentanyl/ *para*-fluorofentanyl; methoxyacetylfentanyl vs ocfentanil; butyrfentanyl vs 4-fluorobutyrfentanyl) resulted in faster CL_{int} but otherwise had little effect. Differences in drug properties, often unknown to users, will result in unpredictable clinical effects increasing overdose risk and complicating treatment. The extended duration of whole-body clearance, in comparison to fentanyl, could potentially affect the effectiveness of overdose treatments e.g. naloxone. Its short half-life, coupled with these longer clearing opioids, may increase the risk of relapse.

Novel "Pyrrolidino" Nitazene Analogues: The New Wave of Novel Synthetic Opioids Identified in Postmortem Cases

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Abstract

Introduction: New subclasses of novel synthetic opioids have been increasingly identified since the 2018 scheduling of fentanyl analogues, beginning soon after with the first emergence of nitazene analogues. Isotonitazene (2019) was the first nitazene analogue to emerge and proliferate, followed by waves of metonitazene (2020), etodesnitazene (2021), *N*-pyrrolidino etonitazene (2022), and protonitazene (2022). Many of these novel opioids are still present in 2023, yet several novel nitazene analogues have also appeared on the recreational drug market. *N*-Desethyl isotonitazene was discovered in drug products without isotonitazene, followed by the identification of other novel "pyrrolidino" nitazene analogues found in authentic biological specimens, including *N*-pyrrolidino protonitazene and *N*-pyrrolidino metonitazene. The rapid identification of emerging nitazene analogues remains a challenge for forensic toxicologists, as their turnover can be rapid and they are frequently present at low concentrations or with their structural isomers (e.g., isotonitazene and protonitazene).

Objectives: This study sought to develop an appropriate analytical workflow involving a standard addition approach for the quantitation of nitazene analogues in blood recently discovered in forensic casework. This was accomplished by developing and validating a new method for five nitazene analogues using liquid chromatography tandem quadrupole mass spectrometry (LC-QQQ-MS). The assay was applied to authentic cases suspected to contain nitazene analogues, either through comprehensive drug screening or scene findings/history.

Methods: Novel nitazene analogues included in this method were *N*-pyrrolidino protonitazene, *N*-pyrrolidino isotonitazene, *N*-pyrrolidino metonitazene, *N*-desethyl isotonitazene, and *N*-desethyl protonitazene. Isotonitazene-D7 was used as the internal standard. The quantitative range was assessed from 0.2 to 50ng/mL. Standard addition was performed using up-spike concentrations of 0.2, 2.0, and 20ng/mL. Samples (0.5mL) were prepared using a basic liquid-liquid extraction. Quantitation was performed using a Waters Xevo TQ-S Micro LC-QQQ-MS. Chromatographic separation was achieved on an Agilent InfinityLab Poroshell C-18 120 (2.7µm, 3.0x100mm) analytical column using gradient elution. Mobile phase compositions were 0.1% formic acid in water and 0.1% formic acid in methanol. Flow rate was 0.4mL/min. Injection volume was 5µL. Method validation was modeled based on the ASB standard. Authentic samples collected for-cause and provided in collaboration with NMS Labs were subjected to the method. Basic demographic and case information were available.

Results: The method was successfully validated and applied to cases screening positive for *N*-pyrrolidino protonitazene or *N*-pyrrolidino metonitazene. Nine postmortem cases collected between December 2022 and March 2023 were submitted for testing. Individuals ranged from 25 to 74 years and were primarily male (75%). Cases originated from Illinois (50%), West Virginia (38%), and Minnesota (13%). *N*-Pyrrolidino protonitazene was confirmed in all samples (concentration mean: 0.9±0.43ng/mL, median: 1ng/mL, range: 0.1-1.5ng/mL). *N*-Pyrrolidino metonitazene was confirmed in five samples (concentration mean: 0.46±0.14ng/mL, median: 0.48ng/mL, range: 0.25-0.63ng/mL). One sample was unable to be quantitated due to low sample volume and was reported qualitatively. *N*-Pyrrolidino protonitazene and *N*-pyrrolidino metonitazene were often found alongside other nitazene analogues (78%) with the presence of metonitazene (n=6, mean: 5.9±6.4ng/mL, range: 1.1-15ng/mL) and protonitazene (n=6, mean: 6.1±8.2ng/mL, range: 0.63-24ng/mL) being most frequently identified. These drugs were found with other novel psychoactive substances (56%), including bromazolam, *para*-fluorofentanyl, desalkylgidazepam, and desalkylflurazepam, and traditional drugs, including fentanyl (89%), psychostimulants (56%), and benzodiazepines (33%). Frequent adulterants in this case series were quinine (89%) and xylazine (67%).

Discussion: Our new nitazene analogue assay was successful in quantifying low levels of *N*-pyrrolidino protonitazene

and *N*-pyrrolidino metonitazene in medicolegal death investigations. These new “pyrrolidino” nitazene analogues were detected alongside other opioids and novel benzodiazepines, further proving the dynamic illicit drug supply. Pharmacological data regarding these novel drugs are currently unknown, but the indication is that these drugs may retain high potency and the ability to proliferate in the drug supply.

S25

The Brothers Butonitazene: Connecting the Dots

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Abstract

Introduction: Since 1956, the Miami-Dade Medical Examiner Department (MDME) has provided accurate, dignified, and compassionate professional death investigative services to the residents of Miami-Dade County, FL. The reliance of autopsy, medicolegal death investigation, and toxicology testing to accurately establish cause and manner of death is often discussed in lectures around the world. At the MDME, the inter-disciplinary coordination within the department applies to every medical examiner case.

Two middle-aged brothers (52 and 55 years) were found dead within one month of each other. The circumstances surrounding their deaths were eerily similar; however, the connection between the two was originally unknown. Through thorough investigation and additional toxicology testing beyond the routine, a connection was revealed that influenced and altered their causes of death.

Objectives: The objective is to discuss the circumstances of the two cases in which collaborative investigative efforts led to the detection of Butonitazene, and ultimately, an accurate classification of both deaths.

Methods: A Shimadzu Nexera X2 ultra-high performance liquid chromatograph coupled to a model 8060 triple quadrupole mass spectrometer was used for the qualitative identification of Butonitazene and the quantitative reporting of other analytes identified.

An Agilent model 6890 gas chromatograph coupled to a model 5973 single quadrupole mass spectrometer was used for the qualitative identification of analytes identified in the paraphernalia found on scene.

Results: Initial toxicology testing in Brother 1 revealed the following analytes in peripheral and central post-mortem blood: Fluoro Fentanyl, Fentanyl (19 ng/mL), 3,4-Methylenedioxy Methamphetamine (MDMA), 3,4-Methylenedioxy Amphetamine (MDA, 64 ng/mL), 7-Aminoclonazepam, Cocaine (<10 ng/mL), Levamisole, and Benzoylecgonine (64 ng/mL).

Brother 2 had already been cremated prior to the MDME establishing jurisdiction, so toxicology testing was only able to be performed on the drug paraphernalia that was found on the scene, which identified the following analytes: Butonitazene, Fentanyl, and Cocaine.

Cause of Death: Combined Drug Toxicity Including Fentanyl and Butonitazene

Additional nitazene Testing for Brother 1 identified Butonitazene.

Cause of Death: Combined Toxic Effects of Cocaine, Methylenedioxy Methamphetamine, Fentanyl, Fluoro Fentanyl, Butonitazene, and Clonazepam

Discussion: The decedents had many things in common besides genetics. Thorough and collaborative investigation of social and medical history revealed similarities in not only their drug use, but also their recent mental health struggles. A review of the brothers' medical records revealed how ill-equipped the healthcare system is at diagnosing and adapting to trends involving novel psychoactive substances.

Fortunately, the same MDME forensic pathologist performed both autopsies. The due diligence of this medical examiner and the rapport he developed with the family of the two brothers exposed important details regarding their drug use that would otherwise remain unknown. Untested drug paraphernalia recovered during the investigation and stored in a local police department vault was transferred to the MDME Toxicology Laboratory for testing. The presence of Butonitazene in the paraphernalia for Brother 2 led to the assignment of additional testing for nitazenes and subsequent detection of butonitazene in Brother 1.

The nitazene class of compounds are an infrequent occurrence in MDME casework and have been identified in only 10 cases since 2022. Proper certification of the deaths of the brothers only occurred because of the collaboration between the death investigators, pathologist, and toxicologists.

These cases highlight the necessity of inter-department cooperation and compassionate work product, delivering an invaluable service to bereaved next-of-kin.

Emergence of the Novel Opioid N-Desethyl Isotonitazene in the Recreational Drug Supply

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Abstract

Introduction: The dynamic conditions surrounding novel psychoactive substances (NPS) continue to challenge the analytical and interpretive efforts of forensic toxicologists. Since the first identifications of NPS decades ago, the landscape of new drugs in the United States has continued to change rapidly, often initiated by national or international drug scheduling. The reaction to these control actions often are simple to complex chemical modifications spawning new drugs. The same is true for the recent evolution of the novel synthetic opioid subclass of nitazene analogues from the emergence of isotonitazene in early 2019 to the most recent emergence of N-desethyl isotonitazene in late 2022. While a metabolite of isotonitazene, N-desethyl isotonitazene itself has recently been encountered as a parent drug. In vitro pharmacological data show that N-desethyl isotonitazene is approximately 20 times more potent than fentanyl. The continued proliferation of high potency opioids has become of significant public health and safety concern, requiring maintained surveillance and response efforts to combat their impacts.

Objective: This presentation will focus on known information about N-desethyl isotonitazene, including cases where the drug was found in drug materials from market surveillance, a urine sample from a drug treatment program, and postmortem blood and urine samples from medicolegal death investigations.

Methods: Initial forensic analysis was performed via liquid chromatography quadrupole time-of-flight mass spectrometry (LC-QTOF-MS) using a SCIEX TripleTOF 5600+ and a SCIEX X500R. Biological samples were prepared using a basic [pH] liquid-liquid extraction. The resulting datafiles were compared against an in-house library database of more than 1,000 analytes. Comprehensive drug screening resulted in qualitative identification for N-desethyl isotonitazene and characterization of other drugs in the samples. Secondary analysis was performed via liquid chromatography tandem quadrupole mass spectrometry (LC-QQQ-MS) using a Waters TQ-S Micro. Quantitative confirmation was completed using a previously validated method for ten nitazene analogues. Drug materials were initially prepared by solvent dilution and analyzed qualitatively using LC-QTOF-MS, followed by basic extraction and quantitative analysis by gas chromatography mass spectrometry (GC-MS). Corresponding case histories were compiled when available.

Results: N-Desethyl isotonitazene was first reported on its own in December 2022 after analysis of a counterfeit tablet from Florida; however, retrospective analyses determined that the first identification was observed as early as September 2022 in a urine sample collected from an individual in Philadelphia. Ten drug material samples collected in Philadelphia from late 2022 through early 2023 tested positive for N-desethyl isotonitazene (estimated purity: <1%) alongside fentanyl (average purity: 3.3%), xylazine (average purity: 59%), bromazolam, and other substances. To date, four known forensic toxicology cases have been quantitively confirmed to contain N-desethyl isotonitazene at <0.2, 0.82, 5.0 and 5.1 ng/mL. Interestingly, some of that casework had combinations of N-desethyl isotonitazene with the NPS benzodiazepine bromazolam, fentanyl, and/or xylazine; combinations that were also observed in the tested drug materials. In a case from New Jersey, an individual with a history of drug use and prior drug related incidences was found unresponsive in bed. Investigators found a mirror with white residue near the decedent. Following autopsy and toxicology testing, the manner of death was ruled accident and the cause of death was attributed to multiple drug toxicity including N-desethyl isotonitazene (0.82 ng/mL), bromazolam, cocaine, methamphetamine, and ethanol.

Discussion: The novel synthetic opioid market continues to transform and evolve, as predicted based on prior experience with NPS. N-Desethyl isotonitazene is a highly potent opioid and one of the newest nitazene analogues to emerge in fatal drug overdoses. Data from drug materials and forensic toxicology casework show that this drug is impacting public health and that testing is necessary in overdoses containing or suspected to contain this new drug.

S27

Fluorexetamine and 2-fluoro-2-oxo-phenylcyclohexylethylamine: New Dissociative Hallucinogens in Forensic Toxicology and Drug Chemistry Casework

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Abstract

Introduction: Novel psychoactive substances (NPS) are emerging designer drugs or research chemicals that are either newly synthesized chemicals or analogs of established drugs designed to mimic the effects of controlled drugs. Like conventional drugs, they can be classified into subclasses based on their chemical structure or physiological effects. NPS arylcyclohexylamines (ACHs) are ketamine and phencyclidine (PCP) analogs that contain a cyclohexane ring attached to phenyl and amine groups on the same ring atom, and act as antagonists on the N-methyl-d-aspartate (NMDA) receptor. They therefore distort sensory perception and are consequently referred to as hallucinogenic dissociatives. The structural diversity of novel ACHs continues to grow with fluorexetamine (FXE) and its positional isomer 2-fluoro-2-oxo-phenylcyclohexylethylamine (2-fluoro-2-oxo PCE) recently being identified in casework.

Objective: Here we report the analytical identification and separation of FXE and 2-fluoro-2-oxo PCE by the Cuyahoga County Regional Forensic Science Laboratory's (CCRFSL) Toxicology Unit. We discuss the challenges that arise when forensic toxicology laboratories are faced with NPS drugs that also have an emerging isomer. Additionally, we present CCRFSL Drug Chemistry Unit's incidence data on these isomers to date. Given that the current literature on FXE and 2-fluoro-2-oxo PCE is limited, we aim to bring attention to their existence and emergence.

Methods: The toxicology cases were subjected to comprehensive toxicology testing which includes, but is not limited to, basic drug screening in blood and/or urine by full scan gas chromatography/mass spectrometry (GC/MS) following solid-phase extraction. Data analysis and analyte detection was achieved via probability-based library matching with the Scientific Working Group for the Analysis of Seized Drugs (SWGDRUG) and the Cayman Chemical Company mass spectral libraries. Thereafter, the relative retention times of the analytes were compared to that of certified reference standards to meet the laboratory's qualitative reporting criteria. Similarly, Drug Chemistry cases were analyzed by GC/MS and identification was achieved by spectral and retention time matching.

Results: The first detected instance of these particular isomers in Cuyahoga County was in postmortem toxicology with GC/MS identification as FXE. A subsequent postmortem case and a driving under the influence of drugs (DUID) case also found signals identified as FXE. The corresponding Drug Chemistry result for one of the cases included a 2-fluoro-2-oxo PCE identification in pills that were coded to be oxycodone HCl 30mg. The DUID case, where blood was tested, was positive for an array of drugs, none of which met the current Ohio Revised Code's DUID per se limits. However, part of the evidence items seized in this case included a bag of white powder labelled "Fluorexetamine". Upon Drug Chemistry testing, the powder in the bag was identified as 2-fluoro-2-oxo PCE. Thirteen further incidences of 2-fluoro-2-oxo PCE from 10 cases between December 2022 and May 2023 were identified by the Drug Chemistry Unit.

Discussion: Positional isomers such as 2-fluoro-2-oxo PCE and FXE increase the risk of misidentification and subsequent underreporting particularly if the GC/MS relative retention time is not obvious or is distorted by the intensity of the peak. Our observations thus far are that compounds in this NPS drug class are frequently co-occurring with fentanyl, and/or designer benzodiazepines. Our experience stresses that the dynamic nature of the NPS climate calls for vigilant screening and identification protocols to avoid overlooking NPS drugs.

PLATFORM

First Identification of (±)-*cis*-Δ^8- and Δ^9-Tetrahydrocannabinol in Biological Specimens by One-Dimensional UHPLC–MS/MS

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Abstract

Introduction: Tetrahydrocannabinol (THC) isomers and derivatives continue to grow in popularity due to the increasingly complex legal status of *Cannabi*s herbs. Their presence in forensic toxicology samples creates challenges in postmortem, drug-facilitated crime, and impaired-driving casework. Analytical methods with short run times may misidentify some of these isomers and their metabolites. The Toxicology Unit of the Cuyahoga County Medical Examiner's Office (CCMEO) developed an extended analytical method for detecting several cannabinoids in blood and urine by liquid-liquid extraction and ultrahigh performance liquid chromatography–tandem mass spectrometry (UHPLC–MS/MS).

Objectives:

1. To develop and optimize a method for identifying the most relevant cannabinoids, including 11-OH-Δ^8-THC and 11-OH-Δ^9-THC.
2. To identify additional peaks seen in case samples using this method.

Methods: Sample preparation included hydrolyzing urine samples at 55°C for 30 minutes with a genetically modified β-glucuronidase enzyme. Both blood and urine samples were then pH-adjusted with phosphate buffer and rotated with 9:1 hexane/ethyl acetate solution for 30 minutes. After centrifugation for 10 minutes, the organic phase was retained, evaporated to dryness, and reconstituted with mobile phase for instrumental analysis. Analysis was performed with a Thermo Scientific™ Vanquish™ Horizon LC System coupled with a TSQ Altis™ triple quadrupole tandem mass spectrometer. Baseline resolution was accomplished with a solid-core Kinetex 1.7 µm C18 100 Å 150 x 2.1 mm column. The aqueous mobile phase A was 0.1% formic acid (v/v) in LC-MS grade water, and the organic mobile phase B was 0.1% formic acid (v/v) in methanol. The flow rate was 0.3 mL/min with a run time of 32 min. The column oven and autosampler were kept at 60°C and 5°C, respectively. The Thermo Scientific™ Chromeleon™ 7.2 SR4 and Xcalibur™ 4.4 user interface software controlled the UHPLC. The Thermo TSQ Altis™ 3.2 and TraceFinder™ 5.1 software packages were used for data collection and processing.

Results: The hydrolysis resulted in >95% efficiency, measured by *trans*-Δ^9-THCA glucuronide. The seven-point, linear, 1/x weighted calibration curves had dynamic ranges of 2 to 100 µg/L (11-OH-Δ^9-THC, Δ^9-THCA, 11-OH-Δ^8-THC and Δ^8-THCA) and 1 to 50 µg/L (*trans*-Δ^9-THC, *trans*-Δ^8-THC, both epimers of Δ^{10}-THC, and both stereoisomers of hexahydrocannabinol (HHC)). Δ^9-THC, Δ^8-THC, 11-OH-Δ^9-THC, and Δ^9-THCA were designed to meet quantitative reporting criteria within the dynamic ranges of the calibration curves. All other analytes were designed to meet qualitative reporting criteria. During method optimization, case specimens previously suspected to contain mixtures of THC isomers were evaluated with this method. Surprisingly, some urine specimens contained unexpected peaks with the same mass transitions as *trans*-Δ^8 and *trans*-Δ^9-THC, identified as the corresponding *cis* isomers using relative retention time, two transitions, and ion ratio comparison to reference material.

Discussion: One of the advantages of this method is that urine samples can be assayed simultaneously with quantitative blood batches. To the authors' knowledge, this method is the first to quantify 11-OH-Δ^9-THC in the presence of 11-OH-Δ^8-THC and to identify (±)-*cis*-Δ^8- and Δ^9-THC in biological specimens using one-dimensional UHPLC–MS/MS. In addition, recent studies reported the presence of *cis*-Δ^9-THC in CBD-rich industrial hemp varietals of *Cannabis sativa* L., supporting the isomers' identification in biological samples following hemp-derived product use. Based on our findings, an extended run time may be necessary for confident identification, quantification, and interpretation of emerging THC isomers and metabolites in light of their varying potencies.

References: Tolomeo, F., Russo, F., Kaczorova, D., Vandelli, M. A., Biagini, G., Laganà, A., ... & Citti, C. (2022). Cis-Δ9-tetrahydrocannabinolic acid occurrence in Cannabis sativa L. Journal of Pharmaceutical and Biomedical Analysis, 219, 114958.

S29

Previously Unidentified Major Hydroxylated Metabolite of Cannabidiol

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Abstract

Introduction: Cannabidiol (CBD) is not believed to be psychoactive, but use has increased dramatically in recent years all across the United States due to a number of perceived benefits and an unregulated market. The widespread use as well as a need to differentiate use of products derived from marijuana (cannabis with greater than 0.3 % Δ9-tetrahydrocannabinol, Δ9-THC) from the use of products derived from hemp (cannabis with less than 0.3% Δ9-THC), warrant the analysis of CBD and metabolites in forensically important matrices such as blood and urine. While both 7-hydroxy-CBD and 7-carboxy-CBD are major metabolites of CBD, they were likely identified based on suspected analogy with THC metabolism and studies of urinary metabolites of CBD are lacking.

Objectives: The objective of this study was to use urinary specimens from a controlled CBD dosing study to investigate the urinary metabolites of CBD and verify if the metabolism and excretion is in fact similar to that of THC.

Methods: Urine specimens (n=46) from a CBD dosing study previously analyzed for CBD, 7-hydroxy-CBD and 7-carboxy-CBD by liquid chromatography tandem mass spectrometry (LC-MS/MS) were reanalyzed using liquid chromatography time-of-flight mass spectrometry (LC-QTOF) on a Waters Xevo G2-XS. The specimens selected include specimens with high concentrations of 7-hydroxy-CBD as well as pre-dosing and placebo specimens from the same individuals.

Briefly, sample preparation consisted of dual hydrolysis using both enzymatic and base hydrolysis, solid phase extraction and a 19 min gradient LC method using 0.1% formic acid in water and acetonitrile on a Waters CORTECS Solid Core C18 Column. Non-targeted mass spectrometry data was collected in the MSE mode. Specimens were analyzed both with and without hydrolysis.

The search of metabolites was based on a list of exact masses of theoretical metabolites. Identification criteria included retention time, mass error, presence after CBD administration, and absence in pre-dosing specimens and controls.

Results: With respect to CBD, 7-hydroxy-CBD and 7-carboxy-CBD, relative peak areas between specimens were in agreement with those from the LC-MS/MS, indicating that the sample preparation and instrumental analysis was adequate to detect those metabolites. In addition to previously known metabolites, a number of potential metabolites were identified. The most abundant mono-hydroxylated metabolite was not 7-hydroxy-CBD but a yet unidentified metabolite that eluted prior to 7-hydroxy-CBD at 5.4 minutes. We have also verified that it is not 6a-hydroxy CBD using a reference standard. Similar to THC, CBD in urine is highly conjugated. Results show that a mono-hydroxylated glucuronide in the unhydrolyzed urine, potentially related to either the most abundant unidentified mono-hydroxylated metabolite or 7-hydroxy-CBD.

Discussion: The results indicate that the metabolism and excretion of CBD is different from that of THC, and more importantly that the major urinary metabolite of CBD is unlikely to be 7-hydroxy-CBD, as previously suspected. The unidentified metabolite might be hydroxylated on the side chain, as previous work using a single specimen indicated oxidative reactions on the pentyl sidechain as a major pathway of CBD metabolism. Further efforts should be directed to identify the major hydroxylated metabolite.

PLATFORM

S30

OSAC Forensic Toxicology Subcommittee Development of a Standard Test Method for Ethanol in Blood

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Abstract

Introduction: Over the past several years, the Forensic Toxicology Subcommittee of the Organization of Scientific Area Committees (OSAC) for Forensic Science has been drafting standards and best practices. Once completed within the OSAC, the documents are submitted to the Academy Standards Board (ASB), an ANSI-accredited standards development organization (SDO), to solicit public comments and finalize the documents as American National Standards. Of recent, the Forensic Toxicology Subcommittee has been drafting a standard test method for the identification and quantitation of ethanol in blood. The creation of a standard test method is new to the field of forensic toxicology and has the potential to facilitate consistency amongst laboratories performing blood ethanol analysis.

Objectives: An update on the two documents pertaining to ethanol/volatile(s) analysis will be provided to the forensic toxicology community. This presentation will promote awareness to stakeholders on the developments of new documents within the Forensic Toxicology Subcommittee of the OSAC. Additionally, it will explain the differences between the standard test method and a standard practice including the advantages of having both as American National Standards.

Methods: The standard test method will include the identification and quantitation of ethanol in blood using dual column GC-FID/FID, dual column GC-FID/MS, or single column GC-FID/MS. Within the development of the standard test method, a validation study will be performed that will include round-robin studies to establish the bias and precision of the standard test method. This level of detail will enable laboratories who choose to use the standard test method to verify their ability to use the method at the same level of performance. These verification studies are considerably easier than performing full validation. The standard practice will provide minimum requirements for laboratories performing volatile(s) identification and quantitation. This standard practice will not serve as a standard test method, thereby requiring laboratories to conduct full validation studies of the method to meet *ANSI/ASB Standard 036, Standard Practices for Method Validation in Forensic Toxicology*.

Discussion: The creation of a standard test method for blood ethanol analysis serves multiple purposes within the forensic toxicology community. First, it serves to address the omission of ethanol and other volatiles from the requirements of *ANSI/ASB Standard 113, Standard for Identification Criteria in Forensic Toxicology*. Further, a standard test method for blood ethanol has the potential to facilitate standardization across laboratories throughout the United States. It should be noted that in addition to the standard test method, the Forensic Toxicology Subcommittee is also drafting a standard practice to establish the minimum requirements for acetone, ethanol, isopropanol, and methanol in biological fluids.

PLATFORM

S31

LC-MS/MS Quantification of Δ8-THC, Δ9-THC, THCV Isomers and Their Main Metabolites in Human Plasma

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Abstract

Introduction: In recent years, isomers of tetrahydrocannabinol (THC) and tetrahydrocannabivarin (THCV) gained popularity for recreational and therapeutic applications, but little is known about their pharmacokinetics and metabolism. Cannabis consumption is generally monitored by detection of Δ9-THC and its metabolites, 11-hydroxy-Δ9-THC (Δ9-11-OH-THC) and 11-nor-9-carboxy-Δ9-THC (Δ9-THCCOOH), in blood, plasma or urine. Recently, the surge of preclinical, clinical and observational trials involving Δ8-THC and Δ8-THCV increased the need for sensitive and specific analytical assays to establish dose-effect relationships.

Objective: We developed and validated an online extraction high-performance liquid chromatography coupled to tandem mass spectrometry (LC/LC-MS/MS) assay for simultaneous quantification of 13 cannabinoids and metabolites including Δ9-THC, 11-OH-Δ9-THC, Δ9-THCCOOH, Δ8-THC, 11-OH- Δ8-THC, Δ8-THCCOOH, (6aR,9R)-Δ10-THC, (6aR,9S)-Δ10-THC, Δ9-THCV, 11-nor-9-carboxy-Δ9-THCV (Δ9-THCV-COOH), Δ8-THCV, 11-hydroxy-Δ8-THCV and Δ8-THCV-COOH in human plasma. The novelty of this approach is the simultaneous quantitative analysis of the isomers of THC, THCV and their main metabolites. The assay was designed for application to a controlled clinical administration study.

Methods: Human plasma was fortified with cannabinoids at varying concentrations within the working range of the respective compound and 200 µL extracted using a simple one-step protein precipitation procedure. Two-hundred and fifty µL sample supernatant was injected onto the extraction column (3.0x5.0 mm, 2.7 µm particle size, C8), then the analytes were back-flushed onto the analytical column (3.0x150 mm, 2.7 µm particle size, RP-Amide). Analysis was performed on a Sciex API5000 Triple Quadrupole MS/MS in positive multiple reaction monitoring (MRM) mode. The run time was 17 minutes.

Results: The method was validated according to 2018 FDA guidelines. Linear ranges were 0.5-400 ng/mL or 1-400 ng/mL for all analytes except Δ8-11-OH-THC (2.5-400 ng/mL). Acceptance criteria for intra- and inter-batch accuracy (85-115%) and precision (<15%) were met for all compounds in human plasma. Mean extraction efficiency was 68.8-106% and mean absolute matrix effect ranged 4.2-25.9% in plasma. No carry over was detected. The assay was used to quantify Δ8-THCV and its main metabolites in 534 plasma samples from a double-blind, placebo-controlled study in healthy participants (Advarra Institutional Review Board Pro00059879; approved 20 December 2021). Two-hundred and thirty-six samples were positive for Δ8-THCV, 157 for 11-OH-Δ8-THCV, 383 for Δ8-THCV-COOH, 42 for Δ9-THCV and 260 for Δ9-THCV-COOH.

Discussion: We present a validated, high-throughput, sensitive and specific assay for the simultaneous quantification of the Δ8 and Δ9 isomers of thirteen cannabinoids and key metabolites for clinical monitoring and research studies.

PLATFORM

Prenatal Exposure to Kratom: Development of an LC-MS/MS Assay for Mitragynine and Speciociliatine in Umbilical Cord and Comparison with an Assay for Mitragynine in Meconium

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Abstract

Introduction: Kratom is an herbal drug which is legal in most of the United States. While it is marketed as a safer alternative to opioids, it can cause opioid-like withdrawal symptoms when discontinued after regular use. Several case studies have shown that kratom exposure in-utero can lead to symptoms in newborns consistent with neonatal abstinence syndrome. Kratom testing is currently not widely available clinically, especially for neonatal specimens, for which the range of concentrations observed is not well characterized.

Objectives: To develop and validate a method for the detection of in-utero kratom exposure in umbilical cord tissue and compare concentrations of mitragynine observed in meconium.

Methods: Umbilical cord or meconium are homogenized in solvent, and internal standards are added prior to solid phase extraction (strong cation exchange). Targeted analytes are separated by reverse phase chromatography on a phenyl hexyl column and detected by tandem mass spectrometry (electrospray ionization, positive mode). For umbilical cord homogenate, diastereomeric kratom alkaloids mitragynine (MG) and speciociliatine (SC), are targeted individually with a cutoff concentration of 0.08 ng/g. For meconium homogenate, pre-analytical hydrolysis is also included because the assay is part of a panel containing 49 other drug analytes some of which (unlike MG and SC) can form glucuronidated or sulfated metabolites. In meconium MG is the only targeted kratom analyte and a cutoff concentration of 10 ng/g is used. Validation experiments assessed accuracy and precision in samples spiked with MG and SC, intra-patient precision in authentic positive patient samples, ion suppression, in-matrix and extract stability, LLOQ, linearity, and carryover.

Results: Using the validated methods, residual umbilical cord tissue and meconium specimens submitted for newborn drug testing (ARUP Laboratories) were evaluated for kratom positivity; paired specimens were not used in this study. For umbilical cords tissue, only samples which were positive for at least one opioid analyte were screened, 35 positive specimens exhibiting varied kratom analyte distributions were identified. In 23 samples SC was the most abundant Kratom analyte, in 12 MG was the most abundant. The mean, median and maximum concentrations of MG were: 7.18 ng/g, 0.444 ng/g, and 90.1 ng/g. The mean, median and maximum concentrations of SC were: 12.5 ng/g, 0.613 ng/g, and 169 ng/g. For meconium, 14,860 samples were analyzed and 90 MG positive samples were identified (0.61%). In meconium the mean, median and maximum concentrations of MG were: 1,650 ng/g, 423 ng/g and 20,320 ng/g. In a separate study of drug prevalence in umbilical cord tissue, 1259 samples were randomly selected for analysis from a total of 1748 samples collected throughout the state of Utah; two specimens were positive for kratom giving a calculated prevalence of 0.16%.

Discussion: Umbilical cord and meconium are often considered equivalent specimen types for detecting drug-exposed newborns. While these specimens can be viewed as complimentary sample types, the cutoff concentrations and target analytes may need to be adjusted to achieve similar positivity rates between the specimens. From a sample collection standpoint, umbilical cord is the superior sample type as it is immediately available at birth; meconium in contrast is more difficult to collect and may be unavailable for some births. However, concentrations of hydrophobic drugs such as kratom are significantly lower in umbilical cord than in meconium, making analysis potentially more challenging. In the case of kratom, it was necessary to develop a stand-alone assay for umbilical cord whereas in meconium kratom could be included in a 50-compound drug panel. Comparison of results in paired specimens would help further guide selection of cutoff concentrations and analytes to report.

S33

Performance of an Untargeted LC-HRMS Screening Method for Tier I Substances

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Abstract

Introduction: Forensic toxicology laboratories are increasingly utilizing untargeted liquid chromatography with high-resolution mass spectrometry (LC-HRMS) techniques as screening tools for presumptive substance identification in biological specimens. Adoption of untargeted LC-HRMS technology facilitates sensitive and selective detection of numerous diverse drugs, toxins, and metabolites extracted from complex matrices. Notably, analytical target lists are flexible and dynamic as spectral libraries are readily updated when novel psychoactive substances emerge. Despite the benefits of untargeted LC-HRMS approaches, no one extraction technique and data acquisition method will work well for all analytes.

Objectives: This work aimed to assess the performance of the National Safety Council's (NSC) Tier I substances via a lipid clean-up extraction from whole blood and LC-HRMS screening tool.

Methods: An untargeted analyte extraction approach and LC-HRMS data acquisition method was developed and its performance assessed for Tier I substances. Target analytes (n = 35) were extracted from whole blood using 96-well Agilent Captiva EMR-lipid cleanup plates. A Waters Xevo G2-XS quadrupole time-of-flight (QToF) mass spectrometer with electrospray ionization (ESI) was used in both positive and negative ionization modes. Separation was achieved via a Waters Acquity HSS C_{18} (1.8 µm, 2.1 x 150 mm) column at 50 °C. Positive ion mode separation utilized 5 mM ammonium formate at pH 3.0 (MP A) and acetonitrile with 0.1% formic acid (MP B). Gradient conditions started at 87% MP A, ramping to 50% at 10 min, 5% at 10.75 min, holding at 5% until 12.25 min, returning to 87% at 12.5 min, and holding until 15 min. Negative ion mode separation used 0.001% formic acid (MP A) and acetonitrile with 0.1% formic acid (MP B). Gradient conditions started at 87% MP A, ramped to 5% at 4.5 min, held until 5.45 min, ramped to 87% at 5.5 min, and held until 7.5 min. A data-independent acquisition mode, MS^E, was used to simultaneously collect precursor and product ion data within a single run. MS^E includes a low (6 eV) collision energy to capture parent ions and a higher, ramped collision energy (10-40 eV) to collect daughter ions. Peak detection was performed using the Waters UNIFI 3D peak algorithm and m/z-retention time pairs to match data against an in-house spectral library. Performance measures was carried out for 32 NSC Tier I substances, excluding Δ^9-THC and its OH- and COOH- metabolites due to poor ionization. Performance measures included: extraction recovery, limit of detection, ionization suppression/enhancement, and interferents. Endogenous interferents were assessed by using different whole blood lots and exogenous interferents included analytes of similar structure and other Tier I substances.

Results: Performance measures of 32 analytes were considered and data coalesced according to drug class and chemical structure. Measures from Δ^9-THC and its OH- and COOH- metabolites were excluded due to limited ionization. Limits of detection were all below recommended screening cutoff concentrations, with several sub-nanogram per milliliter LODs. Extraction recoveries varied, ranging from 10% (morphine, buprenorphine) to greater than 100% (oxycodone, temazepam, oxazepam, nordiazepam, lorazepam, clonazepam, and carisoprodol). Ionization suppression and enhancement also varied, with results spanning 42.8% (carisoprodol) to greater than 100% (many). Significant run-to-run variability was detected, and quantified through a sensitivity analysis (range: 2.5-200%, average: 38.6%). No endogenous nor exogenous interferents were identified.

Discussion: The method described in this work is efficient and effective for qualitative screening. That said, it is critical that laboratories establishing untargeted LC-HRMS screening assays must be aware of the strengths and limitations across diverse drug categories and chemical structures. Results from this work will be used to inform future performance quantification efforts of the NSC's Tier II drugs of abuse.

Conflict of Interest

This work is supported by Award Number 15PNIJ-21-GG-04171-COAP, awarded by the National Institute of Justice, Office of Justice Programs, U.S. Department of Justice. This material is based upon work supported by the National Science Foundation Graduate Research Fellowship Program under Grant No. DGE-2137424.

S34

Development of an Automated Sample Preparation Method

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Abstract

Introduction: Advances in technology have resulted in automated instrumentation becoming more accessible in forensic laboratories, including toxicology. However, the application of automated liquid handling has been minimally employed in the field of forensic toxicology. The sustained increase in overdose deaths nationally is a direct contributor to the increase in demand experienced by forensic toxicology laboratories. This continual increase in demand has primed the field for the introduction of an automated, efficient manner of sample preparation.

Objectives: The routine manual extraction method implemented within the laboratory utilizes a protein precipitation, size-exclusion filtration, followed by dry down and sample reconstitution. The goal of this project was to translate the current manual method to an automated sample preparation utilizing the Hamilton Microlab VANTAGE Liquid Handling System.

Methods: In-house method development and programming of the automated liquid handler was performed to ensure the automated method remains similar to the manual method. Multiple pieces of custom labware were designed, allowing for the exploration of various techniques throughout method development. Labware customization and custom integrations allowed for the two methods to remain nearly identical with changes to consumables differing only to adjust for the requirements of the liquid handler.

Results: The development of this sample preparation method resulted in an ASB 036 validated automated sample extraction method that leverages the same principles and techniques originally used by the manual sample preparation method. The Hamilton Vantage has the capability of preparing 96 samples per batch. Included among the samples is a seven-point calibration curve and quality control samples, all prepared by the automated liquid handler. The typical extraction is completed in an hour, with time fluctuating minimally based on the dry down step.

Discussion: The increasing accessibility of automated instrumentation and robotics introduces a unique opportunity to the various disciplines within the field of forensic science. Within the discipline of forensic toxicology, the implementation of automation allows for the mitigation of human error and maximizes the efficiency of repetitive tasks that do not necessarily require human intervention. The fully streamlined workflow process allows for a higher throughput of samples while maintaining robust and reproducible results. The developed method utilizes simple extraction principles allowing for untargeted drug recovery while maintaining effective clean up prior to sample injection.

S35

Assessing the Cross-Reactivity of Cannabinoid Analogs (Delta-8 THC, Delta-10 THC and CBD) and their Major Metabolites in Six Commercial Cannabinoid Urine Screening Kits

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Abstract

Introduction: There has been an exponential surge in the presence and use of cannabinoids since the federal legalization of hemp (Agricultural Improvement Act of 2018). In several states, cannabis has been legalized for medical use, adult recreational use, and decriminalized. This growth is not only attributed to Δ9-tetrahydrocannabinol (Δ9-THC) and cannabidiol (CBD), the most abundant phytocannabinoid components of cannabis and hemp, respectively, but with many other emerging THC analogs. These products can be purchased online readily or found in local shops (gas stations, vape shops, etc.) in various forms (e-cigarettes, edibles, powders, etc.). Structurally, these analogs are similar to Δ9-THC, yet very little information is available about their detectability using commercially available cannabinoid screening kits. Given their structural similarity, these emerging cannabinoid analogs may cross-react with current immunoassay screening methods used for the detection of Δ9-COOH-THC in urine.

Objectives: To evaluate the cross-reactivity of Δ8-THC, CBD and their major metabolites, Δ10-THC and 11-COOH-HHC chiral analogs using six commercially available homogeneous urine cannabinoid screening kits at cutoff concentrations of 50ng/mL and 20 or 25ng/mL.

Methods: Six urine immunoassay kits (Abbott Cannabinoids – Abbott Diagnostics, LZI Cannabinoids (cTHC) Enzyme Immunoassay – Lin-Zhi International, DRI® Cannabinoid Assay and CEDIA™ THC – Thermo Fisher Scientific, ONLINE DAT Cannabinoid II – Roche Diagnostics, and Syva EMIT®II Plus – Siemens Healthineers) were evaluated at 50ng/mL and 20 or 25ng/mL cutoff concentrations. The analysis was performed on an Abbott Architect Plus c4000 (Abbott Diagnostics). Δ8-THC, olivetol, CBD, and their major metabolites, Δ10-THC and 11-COOH-HHC chiral analogs were evaluated. Cross-reactivity was evaluated by preparing each analyte at 20, 50, 100, and 1000ng/mL in urine. Analogs were analyzed at both cutoff concentrations to determine if the analyte cross-reacted at one or both cutoff(s). Analytes that did not cross-react at 1000ng/mL for a cutoff were considered not detectable. If detected, the appropriate concentration was used as the decision point to determine the precision at the immunoassay's cutoff. Precision was assessed using three QC pools of the analyte prepared at -50%(QCN), the decision point, and +100%(QCP), which were analyzed in five different runs (n=3) along with the respective immunoassay's control materials. The total mean (n=15), standard deviation (SD), and percent coefficient of variation (%CV) were calculated for each QC concentration. A decision point was considered valid if the %CV for the QC was ≤20% for each concentration, and the total mean of the QCN and QCP ±2SD did not overlap the mean of the decision point.

Results: Results are summarized in the following table. Each immunoassay kit had varying selectivity and sensitivity depending on the analog.

PLATFORM

Cannabinoid Kit	Abbott		CEDIA		DRI		Lin-Zhi		Roche		Syva	
Analog/Cutoff Conc. (ng/mL)	50 ng/mL	20 ng/mL	50 ng/mL	25 ng/mL	50 ng/mL	20 ng/mL	50 ng/mL	25 ng/mL	50 ng/mL	20 ng/mL	50 ng/mL	20 ng/mL
Δ^8 THC	200 (77)	100 (39)			200 (53)	100 (43)	200 (53)	100 (31)			200 (65)	100 (32)
11-OH-Δ^8 THC	100 (55)	50 (24)	100 (56)	50 (28)	100 (54)	50 (29)	100 (49)	50 (27)	100 (64)	50 (28)	100 (51)	50 (29)
11-COOH-Δ^8 THC	100 (65)	50 (29)	100 (107)	50 (50)	100 (65)	50 (29)	100 (55)	50 (31)	100 (88)	50 (44)	100 (65)	50 (34)
CBD												
Abnormal CBD												
CBDA-A												
6-OH CBD		1000 (28)				1000 (31)		1000 (23)		1000 (17)		
7-OH CBD		1000 (20)				1000 (23)		1000 (19)				
7-COOH CBD												
9(R)-$\Delta^{6a,10a}$-THC	100 (40)	50 (16)	100 (32)	50 (13)	100 (45)	50 (18)	100 (33)	50 (13)			100 (30)	50 (12)
9(S)-$\Delta^{6a,10a}$-THC	100 (38)	50 (15)	100 (42)	50 (15)	100 (42)	50 (18)	100 (34)	50 (13)			100 (33)	50 (13)
(6aR,9R)-Δ^{10}-THC	100 (39)	50 (14)	100 (17)	50 (6)	100 (40)	50 (16)	100 (35)	50 (11)			100 (39)	50 (14)
(6aR,9S)-Δ^{10}-THC	100 (32)	50 (12)	100 (5)	50 (1)	100 (32)	50 (13)	100 (21)	50 (7)			100 (5)	50 (1)
Olivetol									1000 (47)	1000 (38)		
Olivetolic Acid												
9(R)-COOH-HHC	50 (36)	30 (23)	50 (54)	30 (49)	50 (37)	30 (27)	50 (40)	30 (25)	50 (63)	30 (39)	50 (41)	30 (30)
9(S)-COOH-HHC	50 (29)	30 (18)	50 (40)	30 (31)	50 (30)	30 (21)	50 (30)	30 (18)	50 (25)	30 (16)	50 (31)	30 (20)

An empty cell indicates that there was no cross reaction at 1000 ng/mL.

Discussion: This study demonstrates that the Δ8-THC analogs and Δ10-THC analogs cross-react with commercially available immunoassays. 11-OH-Δ8-THC and Δ8-COOH-THC cross-reacted at approximately two times the Δ9-COOH-THC cutoff concentration, whereas Δ8-THC cross-reacted at approximately four times the Δ9-COOH-THC cutoff concentration for select kits. Variable cross-reactivity was observed in the chiral Δ10-THC analogs. The (6aR,9R)-Δ10-THC and (6aR,9S)-Δ10-THC analogs tended to have less cross-reactivity than the 9(R)-Δ 6a,10a-THC and 9(S)-Δ 6a,10a-THC analogs, with (6aR,9S)-Δ10-THC having the lowest cross-reactivity. 11-COOH-HHC chiral analogs cross-reacted approximately at the Δ9-COOH-THC cutoff concentration, however 11-COOH-9(R)-HHC was more sensitive than 11-COOH-9(S)-HHC. 6-OH-CBD and 7-OH-CBD cross-reacted at a high concentration in select immunoassay kits, however the remaining CBD analogs did not cross-react with the kits. Olivetol cross-reacted at a high concentration on the ROCHE screening kit at both cutoffs, however olivetolic acid did not cross-react with any of the kits.

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S36

Analysis of over 250 Novel Synthetic Opioids by LC-MS/MS in Blood and Urine

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Abstract

Introduction: Novel Synthetic Opioids (NSO) are frequently found in both human performance (HP) and postmortem (PM) forensic toxicology cases. The presence of an NSO is known to cause fatal overdoses, both in isolation and during polysubstance use, and results in impaired driving (DUID) or can facilitate a sexual assault (DFSA). Developing a comprehensive NSO method benefits public health, as it can be used to identify trends in potent opioid use to develop prevention and risk management programs.

Objectives: This project aimed to design a comprehensive, routine method for the selective analysis of 261 novel synthetic opioids in blood and urine.

Methods: This method rapidly extracted 150 µL of blood or urine via protein precipitation followed by filtration (Thomson), evaporation, and reconstitution. Separation was achieved with a 12-minute LC-MS/MS method using a Sciex ExionLC™ with a Phenomenex Kinetex 2.6 µm 100 Å F5 100 x 2.1 mm column. Data was acquired on a Sciex QTRAP 6500+ mass spectrometer utilizing an Ion Drive™ Turbo V electrospray ionization (ESI) source operating in positive multiple reaction monitoring mode (MRM). Data processing was expedited with a custom built-in query created in-house that automated processing and enhanced quality assurance.

Results: This method has been validated according to the ASB/ANSI Standard 036. Assessed in blood and urine qualitatively, 261 unique compounds including fentanyl analogues (fentanlogs), nitazenes, and other miscellaneous synthetic opioids. As 59 isomeric target analytes were placed into groups due to co-elution, there were 203 distinct acquired MRMs. Limits of detection are appropriate for DUID, DFSA and postmortem investigations. To demonstrate applicability, 27 proficiency test blood samples received over an approximate 4-year period demonstrated effective detection of target analytes in forensic casework. In blood, there were 126 expected results comprised of 25 unique target analytes that were within the scope of the described method. Additionally, all 647 fatal accidental overdoses within San Francisco in 2022 were retroactively subjected to analysis by this method and new NSO substances were immediately detected upon implementation.

Discussion: This described method is, to the authors' best knowledge, the most comprehensive NSO published method, with the ability to uniquely identify 203 distinct compounds throughout 261 target components, including newly emerging substances such as nitazenes and xylazine. By increasing the analytical scope of NSO via a single method, this technique detects NSO that may have previously gone undetected. The increases in the scope of testing results in pivotal data benefiting many stakeholders who can develop a better-targeted approach response to the complex opioid crisis.

Impact of Blood Preservatives and Anticoagulants on Cannabinoid Quantitative Analysis

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Abstract

Introduction: The expanded use of recreational and medicinal cannabis products, as well the appearance of new cannabinoid derivatives, increases the importance of implementing pre-analytical (sample collection) protocols and recommendations for cannabinoid testing in biological samples. The accurate quantification of cannabinoids in biological samples is critical for providing a correct result interpretation, especially if specific thresholds have been legally set as in drugs and driving cases in certain states. Previous studies have mainly focused on the impact of time and temperature on the stability of delta-9-tetrahydrocannabinol (delta-9-THC) and metabolites in blood samples; however, the influence of the type of blood collection tube has been frequently overlooked, and no studies investigated the stability of delta-8-THC and metabolites in this type of samples.

Objectives: The objective of this study was to evaluate the impact of different preservatives and anticoagulants in quantitative analysis of delta-9-THC, delta-8-THC, cannabidiol (CBD), and their hydroxy (delta-9-THC-OH, delta-8-THC-OH, CBD-OH) and carboxy (delta-9-THC-COOH, delta-8-THC-COOH, CBD-COOH) metabolites in blood samples stored at room temperature (RT) and at 4°C up to one month. The type of preservatives and anticoagulants investigated were: potassium oxalate-sodium fluoride, 3.2% sodium citrate and K2EDTA.

Methods: Pool stability samples were prepared at 3 and 30 ng/mL for all cannabinoids in human whole blood samples from BioIVT (Westbury, NY) with potassium oxalate-sodium fluoride, 3.2% sodium citrate, or K2EDTA. The pool blood samples were mixed in a rotor for 30 min followed by immediately taking three aliquots of 0.5 mL as the time zero reference. The rest of the pool blood samples were transferred to the corresponding VacutainerTM tube with the same preservatives and anticoagulants (gray-top, light blue-top and lavender-top) and stored at RT and at 4°C for 4 days, one week and one month. All stability samples were analyzed in triplicate. The blood samples were analyzed using a fully validated method by liquid chromatography tandem mass spectrometry.

Results: All cannabinoids showed stability issues (from 21% to total loss) at room temperature after one month of storage, regardless of the type of preservative, with citrate blood showing the highest losses and K2EDTA blood showing the lowest losses. At 4°C the most significant stability issues were observed for citrate blood samples. The most unstable compounds were CBD-OH and CBD-COOH at both storage temperatures, with losses from around 40% to 100%. Delta-8 and delta-9-cannabinoids showed similar stability behavior, with delta-8-THC-OH (up to 61% loss at RT, stable at 4°C) being slightly more stable than delta-9-THC-OH (up to 85% loss at RT and 33% loss at 4°C), and delta-8-THC (up to total loss at 3 ng/mL and up to 42% loss at 30 ng/mL at both temperatures) being more stable than delta-9-THC (up to total loss at 3 ng/mL and up to 85% loss at 30 ng/mL at both temperatures). Delta-8 and 9-THC-COOH were the most stable analytes, showing up to 72% loss at RT and 35% loss at 4°C, depending on the preservative.

Discussion: Blood storage at room temperature should be avoided due to the large decrease in cannabinoid concentration that occurs regardless of the blood preservative. K2EDTA and sodium fluoride blood collection tubes achieved better stability than sodium citrate tubes. Overall, delta-8 and delta-9-cannabinoids showed similar stability behavior.

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Application of Liquid Chromatography Quadrupole Time-Of-Flight Mass Spectrometry (LC-QTOF-MS) All Ions Data Analysis to Authentic Blood Specimens

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Abstract

Introduction: Immunoassay (IA)-based drug screens such as enzyme multiplied immunoassay technique (EMIT) and enzyme-linked immunosorbent assay (ELISA) are utilized for their simplicity and rapid results. However, these techniques possess limitations in scope and specificity of analysis. They rely on identifying classes of compounds by targeting analogous chemical structures and cannot ensure positive identifications for all structurally related compounds (i.e., new psychoactive substances). As a result, toxicologically significant compounds may go undetected through conventional IA techniques.

In contrast, high resolution mass spectrometry (HRMS)-based techniques provide versatility and improved analyte specificity and scope of testing within a single analytical run. By using HRMS methods such as liquid chromatography quadrupole time-of-flight mass spectrometry (LC-QTOF-MS), a more comprehensive analytical scope of compounds may be targeted and specifically identified. This approach is advantageous with the recent publication of minimum standards [1] and recommendations [2] for scope and sensitivity of testing in driving under the influence of drugs (DUID) investigations.

Objectives: This study evaluated the application of a validated LC-QTOF-MS drug screen targeting over 200 compounds categorized as Tier I and Tier II drugs from published recommendations [2]. In addition, it compared the utility of IA and HRMS-based drug screening techniques for DUID investigations in whole blood.

Methods: Nine hundred and eighteen adjudicated whole blood specimens previously analyzed by a 10-panel EMIT and a 6-panel ELISA were re-analyzed by HRMS. A newly validated supported liquid extraction (SLE) method using LC-QTOF-MS All Ions data acquisition was used for sample analysis. Whole blood (600 µL) was diluted with 300 µL of 0.1 M acetic acid and centrifuged prior to SLE analysis. The supernatant adsorbed to the SLE sorbent for five minutes, and drugs were eluted with 6 mL of a mixture of n-hexane, ethyl acetate, and isopropanol. The eluent was evaporated to dryness under nitrogen after the addition of 1% acidic methanol and reconstituted in 20 µL of solvent. Each extract was analyzed by positive and negative electrospray ionization, and IA-based and LC-QTOF-MS results were compared.

Results: Eighty percent or greater correlation between HRMS and IA was determined for most drug categories. However, the concordance of results was affected by the sample age and long-term storage. Lower positivity rates were observed for compounds influenced by sample degradation and analyte instability (i.e., cocaine and marijuana metabolites). Positive drug identifications were determined using the following acceptance criteria: retention time within ± 2%, signal to noise ratio > 3, fragment ion ratios within ± 20%, and fragment co-elution score > 60. In addition, average abundance responses were calculated for all Tier I and II drugs to establish positive cutoff values. An additional 709 toxicologically relevant findings were identified by LC-QTOF-MS. The most abundant drug results observed were methamphetamines/amphetamines, cannabinoids, benzodiazepines, and opiates, respectively. Other notable trends were also identified including the use of synthetic cannabinoids, designer benzodiazepines, PCP, and MDMA.

Conclusion/Discussion: This study highlights the utility of using HRMS-based techniques for comprehensive toxicological drug screening. LC-QTOF-MS and All Ions data acquisition was able to confirm IA results and identify other valuable compounds including NPS. This data shows the benefits of HRMS-based screening methods and how they exceed IA toxicological practices.

References: [1] (2021) ANSI/ASB standard 120, first edition, standard for the analytical scope and sensitivity of forensic toxicological testing of blood in impaired driving investigations. AAFS Standards Board, LLC. https://www.aafs.org/sites/default/files/media/documents/120_Std_e1.pdf (accessed January 1, 2022).

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Combining toxicology testing with FST results to improve cannabis impairment classification

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Abstract

Introduction: The relationship between cannabis use and driving impairment is complex because of the unique pharmacokinetic and pharmacodynamic properties of delta-9-tetrahydrocannabinol (THC). With ethanol there is a clear relationship between amount of alcohol consumed, blood concentrations, and effects on driving performance. With cannabis these relationships are much more complex. The relationship between blood THC concentrations and crash risk has not been established. While it is clear that THC can impair driving, there are still uncertainties regarding the universality of such impairment and its time course, the effects of tolerance, and the accuracy of user self-evaluation of safety. A key question remains: how to best identify drivers who are impaired by cannabis?

This presentation focuses on the toxicology results from the University of California-San Diego's Center for Medicinal Cannabis Research recently competed randomized placebo controlled trial evaluating the effect of smoked cannabis on driving performance.

Objectives: The primary objective was to evaluate how various toxicology cutoff concentrations in both blood and oral fluid affected the classification of participants deemed impaired by the field sobriety tests (FSTs) examinations.

Methods: 191 regular cannabis users were randomized to smoke 700 mg of placebo, 5.9% or 13.4% THC cannabis in a double blind manner. Blood, oral fluid and breath samples were collected serially up until 5 hours after smoking. During the study period participants drove a driving simulator and were administered FSTs by trained drug recognition experts (N=11). FSTs consisted of a walk and turn, modified Romberg, lack of convergence, one leg stand, and finger to nose tests. OF was collected using the Quantisal device. Concentrations of THC and related cannabinoids were quantified using isotope dilution liquid chromatography with tandem mass spectrometry.

Correlation between driving performance (SDLP and coherence) and THC concentrations in blood, OF, and breath were determined using Spearman's rho. P-values were adjusted (padj) for multiple testing using the False Discovery Rate (FDR) method. P-values < 0.05 were considered significant.

Results: No correlation ($p > 0.05$ in all cases) was observed between blood, oral fluid or breath THC concentrations and standard deviation of lateral position or coherence on the driving simulator at any of the time points studied. FSTs were significant ($p< 0.05$) for classifying participants into the THC group vs the placebo group up to 188 minutes after smoking. Seventy-one minutes after smoking, FSTs classified 81% of the participants who received active drug as being impaired. However, 49% of participants who smoked placebo (controls) were also deemed impaired. OF showed less of an impact than blood for reclassifying the active drug cohort, while reclassifying a higher percentage of the placebo group as not under the influence of cannabis.

Discussion: The complete lack of a relationship between the concentration of the centrally active component of cannabis in blood, OF, and breath is strong evidence against the use of per se laws for cannabis. In the largest randomized double-blinded placebo controlled trial to date, our data confirm that THC concentrations (and/or metabolites/related cannabinoids) in blood, OF, or breath cannot be used as a sole indicator of impairment.

Adding a requirement of a positive toxicology test to the FST exam slightly decreased the percentage of participants who smoked active drug that were classified as being impaired and under the influence of cannabis, but dramatically decreased the percentage of placebo group subjects that were classified as such.

S40

Evaluation of the field sobriety tests in identifying drivers under the influence of cannabis

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Abstract

Introduction: With increasing medicinal and recreational cannabis legalization, there is a need for validation of effective and unbiased evaluations for determining whether a driver is impaired due to THC exposure. Field sobriety tests (FSTs) are a key component of officer-based evaluations, yet controlled studies following cannabis exposure have reached disparate conclusions. Most studies have consisted of small sample sizes, utilized evaluations conducted by research staff rather than trained officers, and exposed participants to FSTs prior to drug administration. These approaches may reduce test sensitivity and decrease the likelihood of individuals on placebo doing poorly on the measures (i.e., false positives).

Objectives: To examine the classification accuracy of the FSTs, conducted by trained law enforcement officers, with respect to THC exposure.

Methods: As part of a double-blind, randomized clinical trial 184 individuals with a range of cannabis use (minimum of 4 times in the past month) were assigned to smoke cannabis (5.9% or 13.4% THC) or placebo ad libitum, and then evaluated by trained law enforcement officers on field sobriety tests (Walk and Turn, One Leg Stand, Lack of Convergence, Finger to Nose, and modified Romberg) at 4 subsequent timepoints (1 hr 10 min, 2 hr 20 min, 3 hr 10 min, 4 hr 10 min after smoking). Officers determined whether the participant demonstrated "FST-impairment" based upon performance across all measures, and were asked "which treatment do you think the participant received" on a 5-point scale (from "strongly believed… real marijuana" to "strongly believed… placebo").

Results: Officers classified 81.0% of the THC group (2 THC groups were combined since here were no differences between groups) and 49.2% of the Placebo group as FST-impaired at the first evaluation. Both groups demonstrated improved performance on the FSTs at each successive evaluation. There were no significant background differences (demographics, cannabis use history) between Placebo participants determined to be FST-impaired vs. unimpaired. When officers "strongly" or "somewhat" believe participants to have received THC, 92.8% were classified as FST-impaired; one of 41 participants they believed received placebo was FST-impaired. Of all participants classified as FST-impaired (n=128), officers strongly (74.2%) or somewhat (25.0%) believed that they received THC. The THC group had a significantly higher percent of participants with FST clues (failing to adequately perform on that component) on WAT, OLS, FTN, LOC, and total clues (no mROM differences). A significantly higher proportion of the THC group exceeded SFST cutpoints validated for alcohol ($\geq$ 2 clues) compared to placebo (WAT 76.0% vs 56.5%; OLS 58.5% vs 37.1%).

Discussion: Highly trained officers found significantly worse FST performance in THC compared to Placebo groups, and correctly identified a greater proportion of the THC group as being exposed to THC. However, a substantial proportion of the Placebo group performed poorly on the FSTs. Thus, while the tests discriminated at the group level, the classification accuracy for individuals is less clear. Pre-exposing participants to FSTs prior to study treatment likely underestimates the failure rate in non-intoxicated individuals, given the evidence of possible practice effects. The strong concordance between officer estimates of possible exposure to THC and FST impairment, despite this not always matching with actual exposure, suggests that confirmation bias may remain a concern, even among highly trained experts. This reinforces the importance of the current practice of officers not knowing toxicology results prior to determining impairment status.

The current results suggest that 1) field sobriety tests are useful adjuncts, but in the absence of other indicators do not provide strong objective evidence of THC-specific impairment, and 2) additional efforts to validate existing methods and provide law enforcement with new effective tools for identifying impairment are needed.

Acute and chronic oral dosing of cannabidiol (CBD) with and without low doses of delta-9-tetrahydrocannabinol (Δ-9-THC)

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Abstract

Introduction: Hemp products that predominantly contain cannabidiol (CBD) and low concentrations of delta-9-tetrahydrocannabinol (Δ-9-THC) are legal in many jurisdictions and are widely used for health or wellness purposes. Inadequate research has been conducted to determine the threshold of Δ-9-THC dose in these kinds of products that can impact safety or that result in positive drug testing outcomes.

Objectives: Determine the behavioral pharmacology and toxicology of acute and repeated oral cannabidiol (CBD) administration with and without low doses of Δ-9-THC that approximate levels likely to be found in "full-spectrum" retail hemp products.

Methods: Healthy adults were randomized to self-administer 1mL medium-chain triglyceride (MCT) oil that contained 100mg CBD and either 0mg, 0.5mg, 1mg, 2mg, 2.8mg or 3.7mg Δ-9-THC twice daily for 14 days. Pure CBD and Δ-9-THC were dissolved into MCT oil to create drug products. Initial dose exposure occurred during an 8-hr laboratory session during which subjective drug effects, vitals and cognitive performance were assessed and blood, urine, and oral fluid were collected for analysis. Outpatient dosing (14 days) was monitored via video surveillance and additional assessments collected during brief visits on Days 2, 7, 14, and 21 (1-week washout).

Results: Mild subjective drug effects were reported across all dose conditions and none of the Δ-9-THC dose conditions differed from placebo. There was no indication of impairment of cognitive performance in any dose condition and adverse events were rare. Oral fluid testing consistently showed positive test results (4ng/mL Δ-9-THC cutoff), but only for samples obtained within 1-3 hours of dosing with no evidence of accumulation with repeated dosing. Urine testing using a screening cutoff of 50ng/mL and confirmation cutoff of 15ng/mL THCCOOH showed positive results for 14 out of 15 participants assigned to the 2.8mg and 3.7mg Δ-9-THC dose conditions; 12 of which had at least one positive urine test after a single acute dose. Blood Δ-9-THC concentrations were very low (Cmax = 0.99ng/mL and 1.3ng/mL for 2.8mg and 3.7mg Δ-9-THC doses respectively). None of the urine or blood samples obtained after the 1-week washout (Day 21) were positive. Urine and blood testing at 0.5mg, 1mg, and 2mg Δ-9-THC doses are pending analysis.

Discussion: Acute and chronic administration of MCT oil containing 100mg CBD, with and without small doses of Δ-9-THC, produced mild subjective drug effects, but did not robustly impact cardiovascular or cognitive endpoints. To date, it appears that positive urine and oral fluid drug test results are likely after acute and chronic Δ-9-THC doses of 2.8mg and higher. Additional testing at lower doses is necessary and should be completed prior to the conference.

The Society of Forensic Toxicologists' Toxicology Resource Committee 2023 Survey Results

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Abstract

Introduction: The purpose of the Society of Forensic Toxicologists' Toxicology Resource Committee (TRC) is to identify gaps in funding, training resources, and equipment within publicly funded toxicology laboratories and target resources to further the science of forensic toxicology. Local, state, and national partners use toxicology data to adjudicate, assess, and characterize patterns in drug-impaired driving, monitor drug trends, describe and address mortality related to drug overdose.

Objectives: The 2023 survey assessed current practices, barriers to performing testing, and needed resources for laboratories to provide comprehensive toxicology data. The questions were intended to gain additional information on stop testing and associated policies. Stop testing is an approach when a Blood Alcohol Concentration (BAC) was above a specified level no further testing was conducted to identify/quantitate drugs in submitted samples.

Methods: The survey was sent to ~150 toxicology laboratories performing testing for Driving Under the Influence of Drugs (DUID) including Medical Examiner/Coroners offices for fatal/non-fatal crashes.

Results: Of the 96 (~64%) respondents, 45% conducted state toxicological testing, 12% for multiple counties/regions, 31% for a county, 10% for a city, and 2% for multi-states. Approximately 12% conducted DUI for alcohol analysis, 28% DUI for alcohol and drugs, 18% postmortem, 21% postmortem traffic fatalities (crashes/pedestrians), and 20% drug-facilitated crimes. Only ~4% indicated samples were sent to government-funded laboratories to test for drugs other than alcohol (~86% conducted in-house testing), ~1% sent samples to a private laboratory, and 9% indicated "other". The range of alcohol tests was 0-29,300, with 40% impaired driving, 32% postmortem cases, and 28% drug-facilitated crimes. Scope of testing was based on the following: 1) Academy Standards Board standards, 2) determined by the organization, 3) jurisdictional statutes, 4) National Safety Council's Alcohol, Drugs, and Impairment Division's recommendations (i.e., Tier I and Tier II drugs), or 5) laboratory accreditation. With impaired driving cases, ~42% utilized stop testing, ~50% conducted comprehensive toxicological testing, and ~8% only perform alcohol testing.

Discussion: Data was collected regarding the impact stop-testing policies had on "customers" (i.e., law enforcement, prosecutors, etc.). Respondents indicated the following: 1) enhanced penalties for other drugs were not used in their jurisdictions, 2) a desire for comprehensive testing by local law enforcement, prosecutors, and/or states highway safety offices, 3) stop testing is a long-standing approach use by the laboratory, 4) improvement to case turn-around times, 5) laboratories know they are missing impairing substances/data and realize this information would be useful. For those jurisdictions that utilize stop testing, 48% stopped further testing at 0.10 g/dL, 30% at 0.08 g/dL, 2% at 0.05 g/dL, and above ethanol concentrations. The remaining (20%) utilized another value. Of the respondents, 86% indicated that the laboratory does not limit the scope of drug testing based on the drugs detected, however, ~14% did (i.e. based on their jurisdictional statutes or use of immunoassay for screening which would not identify all impairing substances). Turn-around times reported ranged from 3 days (alcohol analysis only) to >300 days. Approximately, 35% felt they had inadequate staff to develop and validate new analytical methods, 15% responded that they had enough staff, 45% indicated there was sufficient staff to develop some methods but not all, and 5% noted that they had staff, but not enough instrumentation.

Conclusions: Forensic toxicology laboratories can utilize the data obtained from this survey to evaluate their caseloads, turn-around times, and staffing, as well as their policies and procedures for stop testing, and have a better understanding of how they compare with other organizations.

Interference of the novel designer benzodiazepine 4'-chloro deschloroalprazolam with alprazolam analysis in toxicology and seized drug DUID casework

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Abstract

Introduction: One major challenge facing toxicology laboratories is emerging novel substances interfering with routine casework analysis, particularly isomers of commonly identified compounds. A novel isomer of alprazolam, 4'-chloro deschloroalprazolam, was identified in both seized drug and toxicological samples in the United States beginning in late 2021. As isomers, the two share ion transitions and have similar physicochemical properties resulting in similar extraction selectivity and chromatographic retention times (RT). Without a method to differentiate these isomers, 4'-chloro deschloroalprazolam may be misidentified as alprazolam in toxicology casework.

Objectives: This presentation will highlight an interim practice implemented by the Houston Forensic Science Center (HFSC) to differentiate the novel benzodiazepine 4'-chloro deschloroalprazolam from alprazolam in casework samples by liquid chromatography tandem mass spectrometry (LC-MS/MS) until the analyte can be added into the scope of a validated analytical method. Additionally, cases will be presented that were submitted to HFSC with seized drug and toxicology evidence indicating the presence of 4'-chloro deschloroalprazolam.

Methods: HFSC screened blood samples from driving under the influence of drugs cases (received 09/2021-05/2023) for benzodiazepines by enzyme-linked immunosorbent assay (ELISA) and confirmed by LC-MS/MS. An ELISA cross-reactivity experiment was performed by fortifying blank blood with alprazolam or 4'-chloro deschloroalprazolam at 20 ng/mL, the cut-off concentration of the ELISA kit's target, oxazepam. Additionally, an interference experiment was performed by comparing LC-MS/MS chromatography and responses of extracted blood samples fortified with both alprazolam (30 ng/mL) and 4'-chloro deschloroalprazolam (10-500 ng/mL).

Results: 4'-chloro deschloroalprazolam was found to meet all acceptance criteria for alprazolam when analyzed using HFCS's LC-MS/MS benzodiazepines confirmation method, including RT. However, 4'-chloro deschloroalprazolam consistently eluted before alprazolam's deuterated internal standard alprazolam-d5, while alprazolam consistently eluted after. 4'-chloro deschloroalprazolam had an ELISA cross-reactivity of 41%, indicating an approximately 50 ng/mL equivalent to the cut-off concentration of oxazepam at 20 ng/mL.
While not baseline-resolved at any concentration, the LC-MS/MS interference experiment demonstrated accurate alprazolam quantitation when 4'-chloro deschloroalprazolam concentrations ranged from 10 to 100 ng/mL. At higher 4'-chloro deschloroalprazolam concentrations, the individual peaks merged becoming indistinguishable.
Over 20 months, HFSC received cases with toxicological (n=4) and seized drug (n=4) evidence indicating the presence of 4'-chloro deschloroalprazolam. Of the toxicology cases, one had peaks indicating the presence of both alprazolam and 4'-chloro deschloroalprazolam, while the others had a single peak indicating the presence of only 4'-chloro deschloroalprazolam. Two cases suspected of containing only 4'-chloro deschloroalprazolam also had submitted seized drug evidence. While the seized drug evidence for those cases was described as alprazolam by the submitting agency, analysis of the tablets indicated the presence of only 4'-chloro deschloroalprazolam and another benzodiazepine derivative, phenazolam.

Discussion: Emerging designer benzodiazepines, especially novel isomers of routinely-analyzed benzodiazepines, pose analytical challenges for toxicology laboratories. It is important to stay informed of emerging substances and how they may interfere with analysis of commonly targeted analytes. The novel alprazolam isomer, 4'-chloro deschloroalprazolam, has similar chromatographic and mass spectral properties and may be misidentified as alprazolam in toxicology casework. Though developing methods to identify novel isomers may not be practical, alternative approaches can differentiate target analytes from novel isomers. HFSC was able to distinguish alprazolam and 4'-chloro deschloroalprazolam based on their elution order relative to alprazolam-d5, and only report alprazolam-positive cases if the analyte peak elutes after the internal standard peak. If the analyte peak elutes before the internal standard, the laboratory issues a report notifying the stakeholder the result could not be reported due to an interference in the sample.

Impact of drug-driving legislation in England & Wales on fatal road traffic collisions following drug use

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Abstract

Introduction: Drug-driving legislation was introduced on March 2nd 2015 in England and Wales to regulate drug use by drivers. Threshold limits were set for blood quantifications of 8 illicit and 9 prescription drugs deemed to have potential to impair driving.

Objectives: We sought to evaluate the impact of the March 2015 drug-driving legislation on fatal road traffic collisions (RTCs) related to drug use.

Methods: A retrospective study design was used to identify fatal RTCs which occurred in England and Wales in the five years pre- and post-introduction of the legislation (i.e., 2nd March 2010 to 1st March 2019) which had been reported by coroners to the National Programme on Substance Abuse Deaths database. Text detection of relevant terms (e.g., 'car', 'motor*') was performed to identify cases. Cases were excluded when the deceased was not the driver of a motorised vehicle, or the drugs detected at post-mortem had been administered by emergency services to treat the involved parties (e.g., fentanyl, ketamine). Non-fatal RTCs were not studied.

Data analysis and statistics (Student's t-test; Chi squared) were performed using IBM® SPSS™ Statistics for Windows version 27 and Microsoft Excel 365. All percentages were rounded to 0 d.p.

Results: 84 cases were identified where the decedent was the driver of a motorised vehicle (car, motorcycle, van) and psychoactive drugs had been administered prior to the collision were detected at post-mortem; 47 occurred in the five years before (2nd March 2010 – 1st March 2015) and 37 occurred in the five years after (2nd March 2015 – 1st March 2019) introduction of the legislation, representing a reduction of 21%.

210 individual drug detections were made across the 84 cases, with quantifications performed in 58% of detections (n=122/210). Quantifications of drugs with defined limits in the legislation did not increase following its introduction (quantifications performed in 48% of detections pre-legislation [n=39/82], 51% post-legislation [n=33/65], X2 p>0.05), and the number of quantifications over the blood level limits in the legislation decreased (pre-legislation 87% of quantifications over the legislated limits [n=34/39], post-legislation 76% [n=25/33]) although not significantly (X2 p>0.05). At the same time, alcohol was quantified in 98% of detections (n=40/41; an alcohol quantification was requested in the one case where it was not provided, however there was insufficient blood sample available for this further analysis).

Alcohol was implicated in 47% of deaths where it was detected at post-mortem at a level above the limits in the legislation (n=15/32), cannabis in 47% of deaths (n=8/17), and cocaine in 10% of deaths (n=1/10).

A single drug was detected in 24% of cases (30% pre-legislation, n=14/47; 16% post-legislation, n=6/37), with cannabis the most detected single drug (65% single drug cases; 64% pre-legislation, n=9/14; 67% post-legislation, n=4/6). Poly-drug use was evident in 76% of cases (70% pre-legislation, n=33/47; 84% post-legislation, n=31/37). Alcohol with cannabis and/or cocaine were the most detected combinations (39% of cases, n=33/84).

Discussion: Following introduction of the drug-driving legislation in England & Wales, the frequency of fatal RTCs decreased. However, the proportion of drugs included in the legislation subjected to quantification following detection did not change, remaining at approximately 50%. This suggests that the legislation has not changed coronial practice when requesting toxicology tests for fatal RTCs following drug use. Furthermore, when quantifications were requested and legislated drugs were found to be over the stipulated limits and therefore deemed to have the potential to impair driving,

they were implicated by the coroner in causing death in only a small proportion of cases. This may be due to a differences in understanding and interpretation of the types and levels of drugs which can impair driving between coroners and criminal legislators.

S45

The Prevalence of Drugs in Motor Vehicle Fatalities in Jefferson County, AL (2017-2022).

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Abstract

Introduction: Motor vehicle fatalities account for around 10% of the deaths investigated by the Jefferson County Coroner /Medical Examiner's Office (JCCMEO) each year. Drugs play a prevalent role as contributors to these cases whether the subjects are drivers or pedestrians. Both ANSI/ASB and the National Safety Council (NSC) have recommendations for drugs that should be evaluated in fatal driving cases. These include central nervous system stimulants and depressants, cannabinoids and narcotic analgesics.

Objectives: The goal of this research was to review demographic data, case information and drug trends from 2007-2022 in Jefferson County, AL in motor vehicle related fatalities. Drug analyses were evaluated in line with the recommended ANSI/ASB and NSC standards.

Methods: The JCCMEO sample database was search for all cases involving road traffic fatalities from January 2017 to December 2022. The UAB Forensic Toxicology Laboratory analyses all postmortem cases from Jefferson County AL. The samples are analyzed for ethanol, prescription and illicit drugs. Blood and vitreous ethanol results were obtained using headspace gas chromatography with flame ionization detection. The limit of quantitation (LOQ) for ethanol was 0.01g/dL with a linearity range from 0.01-0.4 g/dL. Drug screening was conducted by enzyme multiplied immunoassay technique on urine with confirmation on preserved blood by gas chromatography-mass spectrometry. For all drugs the LOQ was 25 ng/mL with the exception of fentanyl (LOQ of 2.5 ng/mL)

Results: A total of 619 driving related fatalities were analyzed. 443 (~72%) of cases were male and 176 (~28%) were female. 220 (48.6%) of the decedents were black and 193 (45.4%) were white. The demographic of the remaining 6% of cases were Hispanic, Asian, and Other (5.3%, 0.48%, and 0.16% respectively). There were six categories of case, multi vehicle (N=238), single vehicle (N=213), pedestrian (N=145), train (N=10), bicycle (N=9) and motorcycle (N=4). The cause of death was accidental in 99% of the cases with 5 cases classified as homicide and 2 as undetermined. Drugs were note as contributing factors in 205 cases (33%). Alcohol was the most prevalent drug detected (N=217 cases) at a mean concentration of 0.162 g/dL (range 0.010-0.460 g/dL in peripheral blood). In vitreous, the corresponding mean concentration was 0.186 g/dL (range 0.01-0.480 g/dL). CNS stimulants were the next most prevalent drug category with methamphetamine being detected in 12.6% of cases (N=78), and cocaine in 10.3% (N=64). Opioids were the next most prevalent class of drugs with fentanyl (N=22, 3.5%), hydrocodone (N=19, 3.1%), morphine (N=17, 2.7%) and methadone (N=10, 1.6%) making the top 10 list. CNS depressants alprazolam (N=18, 2.9%) diphenhydramine (N=17, 2.7%) and trazadone (N=10, 1.6%) complete Jefferson County's top 10 drug list.

Discussion: A wide variety of drugs and metabolites are seen in road traffic fatalities in Jefferson County, AL with ethanol being the most prevalent drug detected in the time period under review. Stimulants are the second most prevalent class of drugs seen followed by narcotic analgesics. The majority of cases involve poly-drug use similar to other case types in the County. While the number of road traffic fatalities was slightly down in 2022, road traffic incidence remains highly problematic in Jefferson County with drug use being a major contributing factor. The laboratory currently screens urine samples (where available), but with the exception of benzoylecgonine, at concentrations above those recommended by the NSC in the 2021 update. The laboratory is compliant with some but not all of the confirmation cutoff levels, some which are achieved through send out testing due to reliance on GC/MS. Plans to update instrumentation, lower LOQs and expand in-house scope are underway.

Fentanyl in DUID Cases - A Comparison of Three Regions: Alabama, Orange County, CA, and Houston, TX

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Abstract

Introduction: In 2020, Alabama led the United States in the number of per capita prescriptions for opioids for the ninth consecutive year, with nearly four million prescriptions issued. In the United States, fentanyl has emerged as the most prolific drug in the ongoing opioid epidemic and has greatly impacted traffic safety in recent years.

Objectives: This study aimed to evaluate fentanyl prevalence and concentrations in blood and oral fluid in driving under the influence of drugs (DUID) cases in three different regions (i.e., Alabama, Orange County, CA, and Houston, TX).

Methods: DUID cases were evaluated over six years, from 2017-2022. Blood specimens were screened by ELISA or LC-QTOF-MS technology. Fentanyl quantitation in blood was performed by liquid-liquid extraction followed by LC-MS-MS. Alabama has collected both oral fluid and blood in DUID cases since 2018. Quantisal collection devices were used to collect confirmation oral fluid specimens during DUID arrests. Oral fluid samples were extracted using dispersive pipette extraction tips and a Hamilton STARLet, a fully automated liquid handling system. Fentanyl in oral fluid was validated qualitatively for use in casework, but semi-quantitative data were collected for research purposes.

Results: Fentanyl positivity in DUID cases increased for most years in this study. The most significant change occurred between 2017 and 2022, where the fentanyl ranking increased from #14 in Alabama and #23 in Orange County to #4. In Alabama, the prevalence of fentanyl DUID cases increased 4-fold in 2022 compared to 2017. Orange County's increase from 2017 to 2022 was 6-fold. In Houston, the increase was approximately 2-fold from 2019 to 2022. The greatest increase for all labs coincided with the start of the COVID-19 pandemic. In 2022, median fentanyl DUID blood concentrations were 4.7, 11, and 4.7 ng/mL in Alabama, Orange County, and Houston, respectively. Most fentanyl cases were polydrug cases (≥90%). Methamphetamine, THC, and alprazolam were the most frequently detected drugs in combination with fentanyl. In Alabama, the detection of fentanyl in oral fluid was comparable to blood. However, 59% and 8.7% of fentanyl-positive cases had concentrations > 20 ng/mL in oral fluid and blood, respectively. Median concentrations were 214 and 7.9 ng/mL in oral fluid and blood, respectively.

Discussion: The opioid epidemic and the rise in fentanyl use have exacerbated the drugged driving problem and resulted in increased fentanyl cases in all three regions. Fentanyl blood concentrations increased over the evaluated time period, likely due to user tolerance. Oral fluid as an alternative or supplemental specimen to blood is an attractive approach for testing fentanyl in DUID cases. This study contributes to understanding recent fentanyl trends and their impact on highway safety.

S47

Results of a Trial Program for a 32-Hour Work Week in a Forensic Toxicology Laboratory

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Abstract

Introduction: Many studies have supported and championed the benefits of a 32-hour work week. Trials in countries around the world have led to the adoption of this schedule across various fields with much success. The decrease in weekly work-related hours correlates with improved morale, lower stress, and better mental health for employees. While counterintuitive, the decrease in hours has been seen to maintain, or even increase, efficiency and productivity. It is important to note that employees do not take a pay cut for this model; their 32-hour weekly pay is the same as their 40-hour weekly pay. This presentation will focus on the Forensic Toxicology section of the laboratory.

Objective: In 2022, the Montgomery County Coroner's Office and Miami Valley Regional Crime Laboratory in Dayton, Ohio began the process of piloting a program to study what changes would need to be implemented to ensure the success of a 32-hour work week. Following months of preparation, the new work week was implemented for a trial run of six months. Productivity and employee wellness were assessed prior to implementation and six months post-implementation.

Methods: Management and staff were inventive in finding new ways to complete their tasks in 20% less time. Workflows were streamlined and new processes were created to reduce the amount of hours needed to complete the same task, at the same level of quality.

Results: Changes were made to how the employees scheduled their work throughout a given week. Extractions were focused on in the early part of the week allowing for paperwork on the fourth day. Some testing was consolidated and extraction time and data processing were optimized. Employee wellness was improved and the average turnaround time for completed cases was maintained. Eight months later the turnaround time had even decreased ~30% from 18 days to 13 days. During the trial period, survey results showed an increase in overall employee wellness. The new workweek model was formally implemented following the trial period.

Conclusions: Toxicology laboratories can successfully transition to a 32-hour workweek while maintaining quality work at 40-hour workweek productivity. This requires buy-in from not only management but every employee in the section. Working together to refine processes, identify hurdles or rate-limiting steps, and welcoming change allows a lab to even increase its productivity. Employee wellness is also improved as staff now have an extra day to pursue exercise, vacations, hobbies, and family time.

S48

An Update on Standards Development Activities in Forensic Toxicology

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Abstract

Introduction: Standards are beginning to impact daily work in the field of forensic toxicology. Through the efforts of both the Organization of Scientific Area Committees (OSAC) for Forensic Science and the Academy Standards Board (ASB), there have been over a dozen forensic toxicology standards published over the last five years. This presentation will provide an update on the current published standards, those nearing completion, as well as standards you should expect to see in the coming years.

Objective: After attending this presentation, attendees will have a better understanding of the status of discipline specific standards pertinent to the field of forensic toxicology.

Impact on the Forensic Science Community: The presentation will impact the forensic science community by creating greater awareness of standards development activities pertinent to forensic toxicology. It will also increase awareness regarding training, tools and resources that support implementation, compliance monitoring, and broader understanding.

PLATFORM

The Organization of Scientific Area Committees (OSAC) for Forensic Science was created to strengthen the nation's use of forensic science by promoting the use of discipline-specific forensic science standards. To this end, the OSAC drafts standards that are forwarded to standards developing organizations (SDOs) that further develop and publish them. The OSAC also reviews published standards and recognizes them on the OSAC Registry, which serves as a central repository of high quality, consensus-based, technically sound standards.

During this presentation, updates related to standards development in forensic toxicology will be presented. These include:

1) standards that have been added to the OSAC Registry:

- ANSI/ASB 017: Standard Practices for Measurement Traceability in Forensic Toxicology;
- ANSI/ASB 036: Standard Practices for Method Validation in Forensic Toxicology;
- ANSI/ASB 037: Guidelines for Opinions and Testimony in Forensic Toxicology;
- ANSI/ASB 053: Standard for Report Content in Forensic Toxicology;
- ANSI/ASB 054: Standard for Quality Control Programs in Forensic Toxicology Laboratories;
- ANSI/ASB 119: Standard for the Analytical Scope and Sensitivity of Forensic Toxicological Testing of Blood in Medicolegal Death Investigations;
- ANSI/ASB 120: Standard for the Analytical Scope and Sensitivity of Forensic Toxicological Testing of Blood in Impaired Driving Investigations;
- ANSI/ASB 121: Standard for the Analytical Scope and Sensitivity of Forensic Toxicological Testing of Urine in Drug-Facilitated Crime Investigation; and
- ANSI/ASB 152: Standard for the Minimum Content Requirements of Forensic Toxicology Procedures

2) published standards from the Academy Standards Board (ASB) that have yet to go through the OSAC Registry approval process:

- ANSI/ASB 098: Standard for Mass Spectral Data Acceptance in Forensic Toxicology;
- ANSI/ASB 113: Standard for Identification Criteria in Forensic Toxicology;

- ANSI/ASB 153: Standard Practices for Proficiency Testing for Forensic Toxicology Laboratories; and
- ANSI/ASB 156: Guidelines for Specimen Collection and Preservation in Forensic Toxicology

3) documents currently in development by the ASB:

- ASB 055: Standard for Breath Alcohol Measuring Instrument Calibration;
- ASB 056: Standard for Evaluation of Measurement Uncertainty in Forensic Toxicology Laboratories and Breath Alcohol Programs;
- ASB 118: Standard for Breath Alcohol Instrument Specifications;
- ASB 122 Best Practice Recommendation for Performing Alcohol Calculations in Forensic Toxicology; and
- ASB 173 Standard for Education, Training, Continuing Education, and Certification of Forensic Toxicology Laboratory Personnel

4) documents currently being drafted at the OSAC:

- Quality Management Systems in Forensic Toxicology Laboratories;
- Standard Method for Blood Ethanol Identifications and Quantitations; and
- Human Factors Considerations for Forensic Toxicology Laboratories

5) priorities for revisions to existing standards, new documents or work products, and other highlights.

Opportunities for supplemental training related to discipline-specific standards will be presented, as well as additional resources and tools designed to facilitate gap analysis, compliance monitoring, and outreach efforts.

Implementation of Consensus-based Standards Made Easier

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Abstract

Introduction: In 2009 the NAS Report highlighted the need for the forensic science community to embrace the adoption of consensus-based scientific standards. Consensus-based standards in forensic science, and in forensic toxicology specifically, have received broad support. Despite support, standards have limited significance until the are implemented and challenges to implementation exist.

There is no *"EASY"* button for the implementation of standards, but there are resources and tools available that make standard implementation easier. With both Forensic Toxicology specific and applicable Interdisciplinary standards currently on the OSAC Registry and additional standards in the development pipe line, forensic science service providers should be aware of available resources that address identified standard implementation challenges.

Objectives:

- Know how to stay informed and participate in standard development.
- Know what standard implementation resources and tools are available and how to access them on the AAFS and OSAC websites.
- Know how to maximize the usefulness of these resources and tools.

Methods: The presentation will review the standard implementation resources and tools that are available at no cost through the NIST-AAFS Cooperative Agreement. Discipline, standard specific training; factsheets to aid the lay person in understanding the purpose, importance and use of standards; and checklists for implementation monitoring/auditing have been developed. The presentation will demonstrate how to access these resources and tools, how to maximize their effective use, and how to use the checklist for multiple purposes. Current download data related to forensic toxicology specific standards and applicable interdisciplinary standards will be provided.

Discussion: Maximize your resources – your organization's personnel and budget - by pressing the *"EASIER"* button and making the most of these no cost resources.

Discipline, standard specific training can be used to meet training-to-competency, continuing education, certification and licensing requirements in forensic toxicology. These trainings can be taken both "live" and "on-demand" offering an organization necessary flexibility. The webinars can be taken by staff outside of forensic toxicology (e.g., quality assurance personnel, cross-training initiatives) and by external customers (e.g. purchasing, legal).

Factsheets have usefulness within the forensic toxicology discipline as a training tool but their greater value may be to educate those outside of forensic toxicology both internal (e.g., quality assurance, purchasing, administration) and external (e.g., customers, legal, policy makers) to the organization.

The standardized but flexible format of the checklist supports both consistent entries and individualized information within an organization and between organizations as well as process management of standard implementation and auditing.

S50

Underreported methamphetamine positives by laboratories following SAMHSA urine drug test reporting guidelines.

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Abstract

Introduction and Objectives: The Substance Abuse and Mental Health Services Administration (SAMHSA) of the department of Health and Human Services (HHS) has mandatory guidelines for reporting urine drug test results for federal workplace drug testing programs. These guidelines are widely adopted by laboratories servicing the non-federal sector also thereby, impacting the reporting of drug tests in a wide variety of settings including workplace, criminal justice and clinical. According to these guidelines the methamphetamine (MAMP) confirmation cut-off is 250ng/mL and for a sample to be reported positive for MAMP, it must also contain amphetamine (AMP) ≥100ng/mL. In the absence of AMP in the sample, it is to be reported as negative for MAMP irrespective of the MAMP concentration. This rule was instituted to avoid reporting false positive MAMP results due to analytical artifacts in the GC-MS procedures used by the laboratories.

In this retrospective study, we evaluated the impact of this rule on MAMP reporting and assessed its applicability in current-day considering several laboratories now use LC-MS/MS, which is not prone to similar issues as GC-MS with the hot inlet resulting in MAMP production in the presence of ephedrine or pseudoephedrine.

Methods: Past one year data of urinalysis (April 2022- April 2023) from criminal justice court-ordered testing was reviewed to evaluate the incidence of unreported MAMP in this population due to the absence of AMP in the samples. The total number of samples subjected to LC-MS/MS, number of reported positives, and the number of unreported positives due to the absence of AMP were assessed to understand the impact on the overall positivity for MAMP.

Results and Discussion: Of the 127,161 urine samples received between April 2022 and April 2023, 94,856 (74.5%) were reported as positive for MAMP and had AMP concentrations ≥ 100ng/mL per the SAMHSA reporting rule. However, 6,724 samples (5.2%) containing MAMP were not reported positive due to the absence of the metabolite AMP in these samples. Concentrations of MAMP in these samples were significant ranging from 250ng/mL to greater than 10,000ng/mL. The current SAMHSA MAMP reporting rule is leading to under-reporting of positives by at least 5% and it should be re-assessed for its applicability and merits. Potential delay in referring individuals to treatment and the increased safety risk to workplace are considerations for re-assessing this rule.

PLATFORM

Is Chat GPT capable of producing court- ready interpretations of toxicology reports ?

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Abstract

Introduction: Artificial intelligence applications are growing in all areas of science. It is not yet known if this is fit for purpose with regards to the interpretation of toxicology results.

Objectives: This study investigated if the free public access AI software Chat GPT was capable of providing court- ready interpretations of toxicology reports.

Methods: The cases selected for the study were those received from a single UK-based coroner in March 2023.

Chat GPT was requested to produce interpretations of the toxicology results at the standard required by a court from a forensic toxicologist. All unique decedent identifiers were removed from the initial report before the case history and results were entered into Chat GPT. A human toxicologist was also asked to produce interpretations for each case. The interpretations by both the human toxicologist and Chat GPT were compared by an independent, blinded toxicologist.

Both the human toxicologist and Chat GPT were then asked to specifically provide an interpretation of the ethanol concentrations and make comment on the interaction of ethanol with the other co-detected drugs.

Results:26 cases were reviewed. 3 had no significant drug findings. These cases featured 1 death following road traffic collision, 3 following intentional self-harm (i.e., hangings) and 4 deaths from likely alcohol excess, 3 from assaults, 1 from suspected drug overdose and 14 unexplained at the time of autopsy.

Chat GPT provided no interpretation of the clinical effects of ethanol or drug levels in any of the 23 cases. Of note, Chat GPT did not interpret the effects of ethanol or drug levels in the road traffic collision case and made no comment on cognition in the hangings. When instructed to provide specific interpretation of ethanol concentration and effects, Chat GPT did produce appropriate responses, but these were not related to the individual cases. When instructed to provide interpretation of the interaction of ethanol and drugs when both were present, Chat GPT failed to provide interpretations of either ethanol and sedatives or ethanol and stimulants.

The human toxicologist provided interpretation of ethanol and drug effects in all cases, including likely contribution to death.

Discussion: When given a non-specific instruction to produce interpretations of toxicology results equivalent to a forensic toxicologist, Chat GTP was unable to fulfil this requirement. If asked specifically about the ethanol levels it can provide an interpretation based on accepted effects, but it is unable to provide further interpretation or relate any interaction with other drugs

It is possible that the open-ended way in which the questions were asked of Chat GPT has prevented it being fully assessed. It is possible that with specific questions, improved interpretations may be possible. This will be assessed with further work.

Although not fit for purpose yet, it is likely that AI will have a role in interpretation in future, but this should not be limited to the consideration of drug combinations and levels alone. Ideally it will integrate the individual pharmacogenetic and demographic profiles of decedents to improve understanding of individual toxicities.

Finding Forensic Evidence: Toxicological Results from Drug-Facilitated Sexual Assault Cases in Eastern Denmark from 2015–2022

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Abstract

Introduction: Sexual violence is a continuous public health concern worldwide. Drug-facilitated sexual assaults (DFSA) are characterized by sexual activities towards a victim incapacitated by intoxicating substances such as alcohol and/or drugs. Whether the victim has consumed the substance knowingly or through covert administration determines the assault as being of either opportunistic or proactive character. However, to reach a conviction in juridical proceedings, it is important to provide evidence that the victim was under the influence of intoxicating substances during the assault. A vast number of drugs have the potential to facilitate sexual assaults and the literature suggests that ethanol, drugs of abuse and benzodiazepines are typically detected in DFSA cases.

Objectives: The objective of this study was to investigate toxicological findings from police reported DFSA cases analyzed between 2015–2022 at the Department of Forensic Medicine, University of Copenhagen.

Methods: Retrospective data was examined from police reported sexual assault cases from eastern Denmark. Inclusion criteria were 1) victims aged ≥15 years and 2) cases with toxicological analysis of samples from blood, urine, and/or hair. Blood and enzymatically hydrolyzed urine samples were analyzed using an automated robotic setup with protein precipitation and/or solid phase extraction followed by screening using LC–TOF-MS with a simultaneous targeted screening by LC–MS/MS for selected analytes. Segmented hair samples were extracted for simultaneous screening by LC–TOF-MS and targeted screening by LC–MS/MS. Positive findings were confirmed and quantified by LC–MS/MS.

Results: 369 police reported sexual assault cases were included and suspicion of DFSA was indicated in 72% of these cases. Most victims were female (97%) and the age distribution ranged from 15–74 with a mean age of 26 years. Samples were collected within 12 hours in 26% of cases with suspicion of DFSA, whereas 59% of cases without suspicion of DFSA had samples collected within the same timeframe. Preliminary results showed that the most frequently detected substances were ethanol (64%), drugs of abuse (57%), opioids (41%) and benzodiazepines (34%). Antidepressants (20%), antihistamines (8%), antipsychotics (4%) and hypnotics (3%) were also detected. Within these groups, some of the most commonly detected DFSA relevant substances were morphine, diazepam, clonazepam, sertraline, cetirizine, quetiapine and zopiclone. Additional data on matrices and time from assault to sample collection will be presented and compared to the positive findings.

Conclusion: The study presents findings from 369 sexual assault cases from eastern Denmark. In a majority of cases, the victims were females in their twenties. Toxicological findings indicate that ethanol, drugs of abuse, opioids, and benzodiazepines are among the most frequently detected substances in Danish cases of DFSA. These findings are similar to findings in other countries and suggest that an opportunistic approach is most common in DFSA.

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P001

Evaluation of the Correlation between LC/TOF Drug Screening and GC-MS Confirmation Results for Cannabinoids at the Indiana State Department of Toxicology

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Abstract

Introduction: Government agencies such as the Indiana State Department of Toxicology (ISDT) are tasked with drug screening and confirmation of cannabinoids in routine casework. High-resolution mass-spectrometry (HRMS) screening instruments, such as LC-QTOF-MS, have proven valuable in handling toxicology case throughput. However, budget limitations and the exclusion of cannabinoids from many of these HRMS screening techniques can pose a challenge. Due to the exclusion of cannabinoids the use of a separate ELISA screening method specifically for one common compound group may compound the costs and complexity of drug screens for cannabinoids. Therefore, it is important to examine the correlation of HRMS screening techniques versus drug confirmation, to evaluate the effectiveness of these techniques, and inform individuals involved in the criminal justice system of testing false positive rates. An LC/TOF screen that includes Δ9-THC-COOH as a test for cannabinoids was evaluated for its robustness, and correlation rate in ISDT casework.

Objectives: The primary objective of this study was to determine rates of true positives and false positives between LC/TOF screening and GC-MS confirmation results for ISDT cases that involved cannabinoids. A secondary objective of this study was to determine the distributions and concentrations of Δ9-THC and Δ9-THC-COOH in ISDT cases from 2019 to 2021. This will allow for a long-term evaluation of the effectiveness of this method for the screening of cannabinoids. This was achieved through in-house SQL database queries, and data analysis based on compound reports of Δ9-THC-COOH on LC/TOF, and drug analysis reports for Δ9-THC and Δ9-THC-COOH on GC-MS.

Methods: An LC/TOF method using a dual basic and acidic liquid-liquid extraction (LLE) was validated according to ANSI/ASB Standard 036 for 54 drugs of abuse. This utilized 600µL of whole blood, including Δ9-THC-COOH at a cutoff concentration of 10ng/mL, for human performance and postmortem cases in the State of Indiana. The instrument used for screening was an Agilent 1260 Infinity Series LC, coupled to an Agilent 6230 TOF LC/MS. Presumptive positive results for Δ9-THC-COOH by LC/TOF in casework samples were reported based on meeting or exceeding an in-house calculated Analyte Score (AS) and batch relative response value (RR). Confirmation and quantitation of Δ9-THC and Δ9-THC-COOH in 1mL of whole blood was performed using solid-phase extraction (SPE) with derivatization on GC-MS, with an LLOQ of 1ng/mL for Δ9-THC and 5ng/mL for Δ9-THC-COOH. The ULOQ for Δ9-THC was 50ng/mL, and for Δ9-THC-COOH it was 100ng/mL. Confirmation was performed using an Agilent 7890B GC System coupled to an Agilent 5977A MSD. SQL queries from a JusticeTrax™ LIMS-plus (ver.3.8.45) database were used to identify ISDT cases. Data analysis was performed using RStudio (ver.2022.12.0).

Results: A total of 6,209 cases between 2019 and 2021 tested presumptive positive for Δ9-THC-COOH by LC/TOF. True positives were 98.92%, and false positives were 1.08%. The probability of a presumptive positive test for Δ9-THC-COOH resulting in a reported value for Δ9-THC was 87.58%. The mean Δ9-THC concentration was 7.0ng/mL, the median was 4.5ng/mL, and mode was 10ng/mL. The mean Δ9-THC-COOH concentration was 51ng/mL, the median was 43ng/mL, and mode was ≥100ng/mL.

Discussion: Based on available ISDT case data, it was determined that a validated LC/TOF method for the screening of cannabinoids based on Δ9-THC-COOH correlated highly with GC-MS confirmation results for Δ9-THC and Δ9-THC-COOH. False positives occurred in samples at the LC/TOF decision point, but below the GC-MS LLOQ. This shows the robustness of this method for the identification of cannabinoids in routine casework. The distribution of cannabinoids in ISDT casework informs forensic toxicologists, lawyers, judges, and the public of the trends and impact of cannabis in the State of Indiana.

PLATFORM

P002

Development of a Novel Xylazine ELISA for the Rapid Screening of Xylazine in Human Whole Blood and Urine Samples

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Abstract

Introduction: Xylazine is a powerful non-opioid veterinary tranquilizer approved by the Food and Drug Administration for use in animals. However, it has been linked to a growing number of fatalities in the evolving drug addiction and overdose crisis when used in combination with other drugs, such as fentanyl. It causes horrific side effects, with severe rotting ulcers that can develop away from the injection site.

There are currently no known commercially available Xylazine ELISAs for screening of human forensic samples.

Objectives: To develop a rapid, one step, competitive ELISA for the screening of Xylazine in human whole blood and urine samples using a simple, sample dilution.

Methods: A competitive immunoassay was employed for the screening of Xylazine. The Xylazine capture antibody was immobilized and stabilized on a 96-well microtitre plate surface. The ELISA is standardised to Xylazine and, if present, in the sample, competes with horseradish peroxidase labelled Xylazine in the conjugate for a limited number of antibody sites on the microtitre plate. Enzyme substrate was then added to allow a colorimetric reaction to occur. Absorbances were read at 450nm and a standard curve was constructed based on these absorbances. Colour intensity is inversely proportional to the concentration of Xylazine present in the sample. The Randox Xylazine ELISA contains a pre-coated microtitre plate and ready to use standard calibration series, along with a conjugate concentrate that requires a one-step dilution. A simple dilution method was applied to both whole blood and urine samples.

Results: Intra-assay precision was evaluated for 12 replicates of each standard level and two control samples within a single run. Results were expressed as CV (%) and values were verified as <10% across all concentrations tested. Cross-reactivity for the Xylazine metabolite, 4-hydroxy Xylazine, was determined as 100%, ≤7% for clonidine and 4-hydroxy clonidine and ≤0.008% for MDMA, methamphetamine and tricyclic anti-depressants.

The analytical evaluation of the ELISA showed the limits of detection as 0.5ng/ml in both whole blood and urine samples (defined as the mean concentration of at least 20 negative samples plus 3 standard deviations). % Recovery was evaluated by spiking a range of concentrations, with values of 94±9% and 81±5% obtained for whole blood and urine respectively.

In a separate study, twelve whole blood samples and six urine samples that had been screened previously using the Randox Xylazine ELISA were confirmed by LC-MS/MS. 100% correlation was observed for all samples with the ELISA and LC-MS/MS methods.

In the absence of specific regulatory guidelines for Xylazine, cut-off concentrations have not yet been established.

Discussion : This rapid, one-step ELISA provides an excellent screening tool for the detection of Xylazine in human whole blood and urine samples. The Randox Xylazine ELISA (Catalogue number XYL10602) provides a valuable, convenient, and cost-effective analytical tool for the rapid screening (within 1 hour) of whole blood and urine samples following minimal sample preparation. This is relevant because Xylazine is not currently identified routinely by rapid, high throughput quantitative immunoassay methodology. The ELISA provides a preliminary analytical test result. A more specific alternative chemical method must be used to obtain a confirmed analytical result.

P003

DoD Cholinesterase Monitoring Program and Detection of Acute Organophosphate Poisoning

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Abstract

Introduction: Organophosphate compounds have a long history of applications in agricultural industry to control pest caused damages on crops. Many of them are potent anti-cholinesterase agents and can cause serious detrimental effects on humans, such as salivation, lacrimation, urination, defecation, gastrointestinal distress, and emesis (commonly summarized by the acronym SLUDGE). Adverse conditions from acute organophosphate poisoning can rapidly deteriorate to severe respiratory failure, which without immediate medical intervention, can eventually lead to death. Therefore, historically organophosphates have been used as poisons for nefarious purposes and become a subject in forensic toxicology studies. For decades, the U.S. Department of Defense (DoD) has recognized the irreversible inhibition on human red blood cell acetylcholinesterase (RBC-AChE) activity as a valuable biomarker for potential exposure to organophosphates. The DoD has established a nation-wide testing network with the DoD Cholinesterase Reference Laboratory (CRL) located at Joint Base-San Antonio, TX and multiple geographically dispersed satellite testing facilities inside the U.S. The program has become a highly versatile tool to offer rapid and inexpensive testing for acute organophosphate poisoning.

Objectives: The purpose of this presentation is to describe a highly efficient screening program by the DoD for organophosphate and other cholinesterase inhibiting substances caused poisoning and the applications in clinical and forensic toxicology.

Methods: The DoD Cholinesterase Monitoring Program utilizes a time modified Michel delta-pH method developed at the U.S. Army Edgewood Arsenal for human RBC-AChE activity testing. In this method, AChE in the RBC sample catalyzes the hydrolysis of the substrate, acetylcholine bromide, *in vitro* to form acetic acid and choline. The RBC-AChE activity is expressed as the rate of pH change (delta-pH/h) in the sample as a result of acetic acid generation. The method is capable to test up to 51 samples every 17 minutes. Testing accuracy is within ± 0.05 delta-pH/h. The testing result is then compared to the individual or the population-based RBC-AChE activity baseline to determine if organophosphate poisoning likely occurred. This testing method is sensitive, easy to operate, low cost, and can be used in field environments. More importantly, the method does not require known chemical structure or fragmentation pattern of the analyte as modern mass spectrometry-based methods do. Thus, the method is particularly useful in screening unknown organophosphate caused poisoning.

Results: The program has performed annual screening of organophosphate poisoning for approximately 15,000 personnel in agricultural industries and chemical defense since early 1970s. It also enabled the federal government to collect a large amount of adult RBC-AChE activity testing data and to establish a population-based normal RBC-AChE activity reference range (0.63 – 0.89 delta-pH/h). With this reference range, the program is able to provide testing for random adults who do not have individual RBC-AChE activity baselines previously established. In addition, the CRL has used strict chain of custody controls and earned the DoD Clinical Laboratory Improvement Program (CLIP) and ISO 15189 dual accreditation. As a result, the program, especially the CRL has the capability to provide both clinical and forensic toxicology screening testing of organophosphate poisoning.

Conclusion/Discussion: Though little known to people outside the Defense Public Health Centers, the DoD Cholinesterase Monitoring Program is a hidden gem with remarkable versatility of providing rapid and sensitive forensic screening testing for organophosphate poisoning.

PLATFORM

P004

Quantitation of 106 drugs in urine using a fast, 7-minute method with high resolution accurate-mass (HRAM) mass spectrometry

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Abstract

Introduction: Drugs of abuse testing in urine is an essential and routine requirement for forensic toxicology labs. With the ever-growing number of abused drugs and increase in overdoses, labs are detecting, confirming, and a wide range of analytes. For this reason, it is of great importance to develop a fast, high-throughput liquid chromatography mass spectrometry (LC-MS/MS) method to accommodate many drugs of different hydrophilicities and chemical structures, while producing baseline separation of isomers. Orbitrap mass spectrometers generate high-resolution accurate mass data that offers improved sensitivity, selectivity, and accuracy for the detection and quantitation of drugs of abuse in biological matrices.

Objectives: To develop a method for quantitative analysis of 106 drugs of abuse with complete sample preparation workflow and a fast, 7-minute LC-MS/MS method by orbitrap technology and triple quadrupole.

Methods: 106 drugs and drug metabolites covering over 10 different drug classes, 15 isomers, and varying hydrophobicities and polarities were chosen based on high frequency testing in forensic labs. Standards were spiked into negative urine spanning a concentration range of 0.05 to 5,000 ng/mL. 200 µL of each sample were extracted using SOLAµ™ SPE plates. Analytes were separated by ultra-high performance liquid chromatography (UHPLC) using a 7-min gradient and C18+ column. The samples were run on 2 different mass spectrometry platforms to highlight the versatility and applicability of the method to suit different laboratory needs. An orbitrap mass spectrometer was used for targeted screening and quantitation with full scan and targeted data dependent MS2 scanning used with an inclusion list for targeted compounds. The samples were also run by triple quadrupole using optimized SRM transitions with the same sample preparation and chromatography. Each sample was injected in triplicate.

Results: Chromatography exhibited ample separation for isomers including codeine and hydrocodone, methamphetamine and phentermine, and morphine, hydromorphone, and norhydrocodone. Limits of detection (LOD) and quantitation (LOQ) were determined for the 106 drugs. LOQ was defined as the back calculated concentration where % difference and % RSD were less than 20%. Lower limits of quantitation ranged from 0.1 to 100 ng/mL meeting ANSI and industry concentration standards. The data shows a wide dynamic range for compounds such as norbuprenorphine which produced a linear calibration curve between 0.5 and 5,000 ng/mL.

Discussion: This fast, quantitative method on both orbitrap and triple quadrupole platforms was used to detect, identify, and confirm 106 high frequency drugs. Due to the robustness of the chromatography and the sensitivity of the mass spectrometers, extremely low LOQ's and wide dynamic ranges of calibration curves were produced.

P005

Development of a Bead Ruptor-based method for rapid toxicological analysis of synthetic opioids in bones

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Abstract

Introduction: In 2021, 75.7% of overdose deaths were due to opioids in the U.S. Many of these deaths were attributed to novel synthetic opioids. Synthetic opioids are particularly dangerous considering that most of them are extremely potent requiring lower concentrations to be toxic. Their analysis in biological samples such as blood and urine is very challenging. In the case of extreme body decomposition, the only matrix available for toxicological analyses is bone tissue. Due to the time-consuming extraction methods and the lack of correlation between drug concentration in bones and in blood, bones are not considered a good forensic toxicology sample.

Objectives: The aim of this study was to develop a faster and more suitable method for the extraction of synthetic opioids from bone samples and subsequent analysis by GC-MS to be applied in the study of blood-bone correlations. To achieve this goal, we are introducing a new animal model – the rabbit.

Methods: Bone samples were chosen according to their histological structure. Rabbit femurs obtained commercially were used for the initial extraction development. For the extraction evaluation, drug-free rabbit bones were fortified with fentanyl, norfentanyl, AP-237, and AP-238 at 1000 ng/g of bones using a methanolic solution containing all analytes. The solution was added to the surface of the bone and let dry. Bones were homogenized using the Bead Ruptor. The parameters for the best homogenization were optimized. The initial extraction procedure was performed using the Bead Ruptor homogenization with solvent followed by evaporation and analysis. For this extraction, 4 mL of the extraction solvent was added to 500 mg of bone samples followed by the homogenization. Three sets of solvents were tested (methanol, 4:1 butyl acetate: ethyl ether with 0.5 mL of ammonium hydroxide, and 3% ammonium hydroxide in 20:80 isopropanol: ethyl acetate). Due to the necessity of extra clean-up steps, the addition of LLE and/or SPE was also evaluated. For the quantitative analysis, the developed GC-MS method was validated following the ANSI/ASB 036 Guideline. Selectivity, linearity, bias, precision, carryover, LOD, LOQ, and recovery were evaluated. For identification of the targeted analytes, the GC-MS analysis was performed in selected ion monitoring mode (SIM) with the designated ions for all analytes.

Results: Method validation was successfully performed. The initial extractions using only the Bead Ruptor presented yields that varied from 57 to 78%, 32 to 68%, and 57 to 76%, using methanol, butyl acetate: ethyl ether ammonium hydroxide, and ammonium hydroxide in isopropanol: ethyl acetate, respectively. However, the chromatograms showed a high baseline and large peaks. The clean-up procedure based on LLE resulted in lower yields, whereas the SPE procedure resulted in better recoveries and cleaner chromatograms, so SPE is the clean-up procedure of choice. The total extraction time (homogenization, extraction, and SPE clean-up) was performed in 2 hours and resulted in yields ranging from 84 to 98%, for all analytes, except norfentanyl with a yield of 30%. The possible lack of volatility of norfentanyl was a probable cause of poor recovery.

Discussion: Rabbits' bones were chosen due to their metabolic similarity with human bones. Rodents do not present similar bone metabolism, and their use as animal models can be the reason for several disparities in the literature data about blood-bone correlations with different drugs. The total extraction of new synthetic opioids from rabbit bones was shorter when compared to the current bone extraction methods. Given the increase in illicit synthetic opioid cases, it is of forensic interest to develop a method to detect these substances in alternative biological matrices, especially to be applied in cold cases.

POSTER

P006

In vivo Toxicity Evaluation of the Russian Homemade Drug Krokodil

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Abstract

Introduction: "Krokodil" is the street name for the injectable mixture that has been used as a cheap substitute for heroin since 2002 in Russia, Ukraine, Georgia, and other European countries. The main psychoactive compound in krokodil is the opioid desomorphine. However, several other compounds were described to be found inside the krokodil mixture, mainly opioid derivatives and reaction by-products. Krokodil is usually prepared at home by the users themselves in a harsh uncontrolled reaction that starts with codeine tablets, bathroom cleaners, gasoline, car battery fluids, iodine tincture, and matchboxes, all of which are easily available in retail outlets, such as supermarkets and drugstores. The resulting product is a light brown liquid that is called krokodil. Krokodil users present several symptoms such as the formation of black ulcers in the injection site, necrosis, limb amputation, jaw osteonecrosis, speech impairment, and many others.

Objectives: The goal of this study was to understand the toxic effects presented by krokodil users using Wistar male rats as the experimental model.

Methods: Krokodil samples were synthesized mimicking the homemade method applied by krokodil users. Samples were analyzed by GC-MS to confirm the presence of desomorphine. Animals were divided into seven groups and exposed subcutaneously to NaCl 0.9% (control group), krokodil mixture free of desomorphine (blank krokodil group), desomorphine 1 mg/Kg, and four different concentrations of the synthesized krokodil (1, 0.5, 0.25 and 0.12 mg/Kg). Animals were treated once a day for five consecutive days. Animals were monitored daily and euthanized 24 hours after the last administration. The toxicity study was performed through the analysis of biochemical biomarkers using a clinical chemistry analyzer, histology assays, and toxicity assays. The toxicity assays were based on the formation of reactive oxidative species (ROS) and consisted of the analysis of reduced and oxidized glutathione (GSH/GSSG ratio) and lipid peroxidation. Krokodil's capacity to inhibit acetylcholinesterase was also evaluated.

Results: The use of krokodil for only a few days showed to cause injury at the injection area, with the formation of necrotic zones. The biochemical results evidenced alterations in cardiac and renal biomarkers of toxicity, namely creatine kinase, creatine kinase-MB, and uric acid. Significant alteration in levels of reduced and oxidized glutathione in the kidney and heart suggested that oxidative stress may be involved in krokodil-mediated toxicity. Although urinary biomarkers such as N-acetyl-β-glucosaminidase evidenced slight alterations, histological analysis revealed only mild alterations in kidney, liver, and lung tissue. Cardiac tissue necrosis was the most relevant finding of continuous krokodil administration. Krokodil also showed to inhibit acetylcholinesterase, explaining some of the krokodil effects that resemble the ones presented by patients intoxicated with organophosphate pesticides.

Discussion: This study contributed to a better understanding of the toxicity of krokodil abuse, mainly the local and systemic toxicological impact of this complex drug mixture on major organs. As opioids are the main active compounds inside krokodil, when patients intoxicated with krokodil arrive in the ER, the first action is to revert the opioid effects. However, due to the formation of several by-products and the high pH and phosphorous concentration inside krokodil, patients need to be monitored for their renal functions and calcium and phosphorous levels in the blood. We hope these findings will be applied to develop an appropriate treatment strategy for the toxicological effects of krokodil.

POSTER

P007

Fatality due to combined toxicity of Xywav® and ethanol

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Abstract

Introduction: Xywav® is a mixed-salt (calcium, magnesium, potassium, and sodium) oxybate solution, referred to as 'lower-sodium oxybate', prescribed to treat symptoms of narcolepsy including sudden muscle weakness and excessive daytime sleepiness. It is a mixed-salt form of gamma-hydroxybutyric acid (GHB). Here we report the accidental death of a 63 year old woman from acute combined drug toxicity (ethanol and GHB).

The decedent was found in a supine position on her bed. In an adjacent bathroom, an open/partial bottle of rum was located on the counter near an apparently empty bottle of Xywav®. An open sports drink bottle with straw inside was located on the bedside table. The decedent had been last known alive approximately 3 hours prior to being found unresponsive, at which time she was napping in her bed. The decedent's past medical history included multiple sclerosis, dementia, seizures, and depression. Her family stated that she had been 'sliding into a depression' over the previous couple of months, consumed ethanol heavily, and sometimes overtook her prescription medications. Per the spouse, she had taken Xyrem®/Xywav® for a few years to help with sleep but had recently also started drinking alcohol to aide in falling asleep. While there hadn't been any concrete suicidal ideations, she had occasionally mentioned not wanting 'to live like this anymore.'

Objectives: Describe the pathology and toxicology findings associated with this fatality.

Methods: Postmortem specimens were analyzed at the Harris County Institute of Forensic Sciences for volatiles, vitreous chemistries, screened for drugs using a 10-panel ELISA screen and GC/MS. GHB testing was conducted by GC/MS/MS.

Results: At autopsy, mild atherosclerosis and pulmonary edema were noted along with acute/hypoxic ischemic neuronal damage, most likely the consequence of respiratory depression. Other neuropathology findings were consistent with inactive multiple sclerosis. In initial toxicology testing, ethanol (0.174 g/100mL in femoral blood, 0.215 g/100mL in vitreous humor), promethazine (<0.10 mg/L), bupropion (0.35 mg/L), levetiracetam, donepezil, and memantine were identified. After completion of initial testing, given the suspicion of the death having been toxicology-related, further review of the decedent's medications revealed Xywav® having been prescribed. Testing for GHB was added; the concentrations were as follows: 510 mg/L (femoral blood), 190 mg/L (vitreous humor), 330 mg/L (urine).

Discussion: The cause of death was attributed to the acute combined toxicity of ethanol and gamma-hydroxybutyrate with multiple sclerosis being listed as a contributory cause. The manner of death was classified as accident. The reported GHB concentrations are consistent with what has been reported in other deaths attributed to GHB toxicity. While there have been previously reported deaths associated with the use of Xyrem® (another pharmaceutical preparation of GHB; sodium oxybate), it is believed that this is the first reported death associated with the use of Xywav®.

POSTER

P008

Confirmation of Cannabinoids in Forensic Toxicology Casework by Isomer-Selective UPLC-MS-MS Analysis in Urine

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Abstract

Introduction: Confirmation of cannabinoid use by forensic toxicology testing in urine has traditionally focused on Δ9-tetrahydrocannabinol (Δ9-THC) with analysis of its major metabolite, 11-nor-9-carboxy-Δ9–tetrahydrocannabinol (Δ9-cTHC) in free and conjugated forms. Legalization of hemp, however, has led to widespread production and sale of cannabidiol (CBD) derivatives with psycho-activity, including Δ8-THC and Δ10-THC isomers. The increasing availability and growing use of isomer derivatives necessitates an expanded scope of cannabinoid confirmation test protocols.

Objectives: We report a quantitative, isomer-selective method of cannabinoid confirmation by liquid chromatography/tandem mass spectrometry (UPLC-MS-MS) for determination of parent-drug isomers (Δ8-THC, Δ9-THC, Δ10-THC, CBD), as well as isomeric metabolites (Δ8-cTHC, Δ9-cTHC).

Methods: Optimum resolution with minimum analytical run time was achieved by employing a high efficiency solid-core particle column (CORTECS UPLC C18+, 1.6µm, 2.1 x 50 mm) eluted with a mixture of 0.1% formic acid in water and acetonitrile. Application of a step gradient, with near isocratic conditions at both the beginning and the end of the run, facilitated resolution for the early-eluting THC metabolite isomers and later-eluting CBD and THC isomers. A rapid method of hydrolysis, dilution, and UPLC-MS-MS analysis was employed for quantitative co-determination of free and conjugated analytes, using stable isotope internal standardization.

Results: Acceptable performance was achieved for the evaluated method characteristics which included: precision, accuracy, carryover, dilution integrity, matrix effects and interference. Casework experience with the isomer-selective method revealed a 14% prevalence of Δ8-cTHC positive cases with case-to-case variability in the relative concentration of Δ8-THC and Δ9-THC metabolites. CBD prevalence was 10% and the parent drugs, Δ8-THC, Δ9-THC, and Δ10-THC, were not detected in any of the case samples. Interference studies with a prior method highlighted a limitation in the scope of traditional confirmation test protocols and a potential for interference from CBD-sourced derivatives. A comparison of Δ8-cTHC and Δ9-cTHC phase two metabolism is also reported and demonstrates parallel excretion-kinetics for the isomer metabolites.

Discussion: The selective confirmation method for Δ8-THC, Δ9-THC, Δ10-THC, CBD, Δ8-cTHC and Δ9-cTHC in urine provides a simple and rapid method that is adaptable for routine, high-volume testing. Optimization of chromatographic separation was essential in selective analysis for the structural isomers due to similar chromatographic properties, especially the metabolite isomers. Application studies showed prevalence of Δ8-cTHC isomer in toxicology casework and reveal a significant concomitant use of Δ8-THC and Δ9-THC. Interference studies with a prior method highlighted a limitation in the scope of traditional confirmation test protocols and a potential for interference from CBD-sourced derivatives. The literature on derivative chemistry, production, and use also emphasize the need for continuing analytical vigilance in discovery of additional cannabinoid derivatives; this may require further expansion in the scope and selectivity of confirmation testing methods for psychoactive cannabinoid use.

POSTER

P009

Design and Distribution of Traceable Opioid Material (TOM) Kits to Improve Laboratory Testing for Opioids and Associated Drugs of Concern

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Abstract

Introduction: Synthetic opioids are often mixed with other drugs including xylazine, benzodiazepines, and stimulants, and can cause overdose deaths. Accurate identification and measurement of these drugs by laboratories is imperative to preventing overdose deaths and mitigating their harmful effects.

Objectives: The Centers for Disease Control and Prevention (CDC) has contracted the manufacture and distribution of Traceable Opioid Material (TOM)® Kits to rapidly improve the detection of current and emerging drugs of concern by laboratories.

Methods: The kits include 1) Opioid Certified Reference Materials (CRM) kits, 2) Fentanyl Analog Screening (FAS and FAS V1-4) Kits, 3) Opioid Polysubstance Materials (OPM) kits, and 4) Emergent Drug Panel (EDP) kits. These kits have been designed by CDC by curating drug data from recent lists distributed by the U.S. Drug Enforcement Administration (DEA), the Center for Forensic Science Research and Education (CFSRE), and the National Forensic Laboratory Information System (NFLIS). The kits are distributed by their manufacturers, free-of-charge, to federal, state, local, and private clinical laboratories to improve their testing capabilities.

Results: More than 3000 kits have been distributed to both domestic and international laboratories.

Discussion: These kits support testing and research endeavors associated with the opioid epidemic by providing access to relevant, quality, materials that are consistent among laboratories.

Disclaimer: The findings and conclusions in this report are those of the author(s) and do not necessarily represent the official position of the Centers for Disease Control and Prevention.

POSTER

P010

Whole Blood Drug Panel Screening in Impaired Driving Investigations: Development of a Rapid Screening Method using Laser Diode Thermal Desorption coupled to mass spectrometer (LDTD-MS/MS).

Serge Auger, Sarah Demars, Jean Lacoursière, Pierre Picard

Phytronix, Quebec, Quebec, Canada

Abstract

Introduction: Impaired driving is a public health and safety concern, and toxicological testing is a critical part of these investigations. The Academy Standards Board (ASB) published a standard stating the minimum requirements for target analytes and analytical sensitivity (ANSI/ASB standard 120, 2021).

LDTD ion source combine to mass spectrometer allows a fast sample to sample analysis (less than 8 seconds/sample) with high-efficiency protonation, resistance to ionic suppression, low sample volume needed, and no solvent/mobile phase required.

Objectives: The objective of this research was to set extraction methods to analyze whole blood samples. The whole blood samples were screened to detect drugs at LOD level defined in ANSI/ASB standard 120 using Laser Diode Thermal Desorption (Phytronix) and tandem mass spectrometry (Sciex), LDTD-MS/MS.

Methods: The drug list and screening cut-offs described by ANSI/ASB standard 120 were used (Blood screen). To get a specific extraction, two drug panels were used with their specific extraction process. Benzoylecgonine, Buprenorphine, Lorazepam, MDA, Methadone, Methamphetamine, Oxazepam, Temazepam and tramadol were grouped into panel 1. Alprazolam, Amphetamine, Carisoprodol, Clonazepam, Codeine, Hydrocodone, Diazepam, Fentanyl, MDMA, Morphine, Nordiazepam, Oxycodone, Carboxy-THC and Zolpidem were grouped into panel 2. Negative whole blood was fortified with standard mixes and extracted using Salt Assisted Liquid-Liquid Extraction (SALLE) and Liquid-Liquid Extraction (LLE) respectively.

The Panel 1 extraction process is performed as follows: matrix is mixed with internal standard, dilution solution and ZnSO4 (1N). SALLE extraction process was performed and 8 µL of the SALLE upper layer were deposited onto LazWell96 plates.

The Panel 2 extraction process is performed as follows: matrix is mixed with internal standard solution and Methyl tert-butyl ether. Upper layer was transferred to a new tube, evaporated to dryness and reconstituted. Six microliters of the extracted samples were deposited onto LazWell96 plates.

The mass spectrometer is operated in positive/negative ionization mode. A flow rate of 6 L/min with air as a carrier gas and a ramp of 6 seconds to 65% laser power with a 2-second hold are used on the LDTD system.

Results: Three-point screening curves (LOD, 2xLOD and 5xLOD) and two QCs (QC-0.5X: 0.5xLOD and QC-2X: 2xLOD) were prepared in negative whole blood and used to validate the method. LOD drug blood screen values reported in ANSI/ASB Standard 120 were used. The peak area ratio of targeted analyte against the internal standard (IS) ratio was used to normalize the signal.

For the inter-run precision/accuracy experiment, each fortified sample set is analyzed in triplicate on five different days. For the inter-run accuracy, %Bias values between -2.5 and 3.3 were obtained and the precision results were lower than 12.1% CV. All QC-0.5X were detected as negative and QC-2X detected as positive.

For the LDTD-MS/MS analysis, instead of studying the autosampler's stability, the wet stability (extracted solutions kept at 4°C for 1 day) and dry stability (extracts on LazWell plate for 60 minutes at room temperature) were evaluated. After the given stability time, calibration curves were analyzed. The precision obtained for standards ranged between 1.3 and 19.2%CV and their %Bias accuracy ranged between -10.0% and 4.8% of the nominal values.

POSTER

Negative whole blood (EDTA-K2) was collected from 6 volunteers. Samples were then spiked at QC-0.5x and QC-2X levels and screened using LDTD-MS/MS method. The method sensitivity, specificity, positive predictive value, negative predictive value and accuracy were verified. Finally, a Kappa evaluation is done to verify the interobserver agreement. An agreement of 100% was reached for all drugs.

Discussion: LDTD combined to a Sciex mass spectrometer system allows ultra-fast (8 seconds per sample) screening of drugs in whole blood.

P011

Biomarker, prescribed and illicit polar drug class analysis in urine: Development of a rapid screening method using LDTD-MS/MS.

Serge Auger, Sarah Demers, Jean Lacoursière, Pierre Picard

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Abstract

Introduction: Analysis of different biomarker, prescribed and illicit polar drug classes in urine (Ethyl glucuronide, Gabapentin, Pregabalin and GHB) are requested for dosage adjustments or for the detection of potential consumption abuse.

Objectives: Extraction methods were optimized to analyze urine samples and were then transferred to an automated liquid handling system for day-to-day use. The urine samples were screened to detect polar drugs using Laser Diode Thermal Desorption and tandem mass spectrometry (LDTD-MS/MS).

Methods: The polar drugs are defined as hydrophile molecules like Pregabalin, Gabapentin, Ethyl-b-glucuronide and GHB. Chosen drugs were spiked in negative human urine. With these polar drugs, few choices of sample preparation were available. Sample dilution (200 times) was used.

The extraction process was performed as follows: 5 µL of urine samples were mixed with 5 µL of internal standard solution (Gabapentin-d10 (10 µg/mL), Ethyl-b-glucuronide-d5 (5 µg/mL) and GHB-d6 (5 µg/mL)) in methanol and 1000 µL of dilution buffer (1mM KH2PO4 in Methanol:Water (60:40)). Six microliters of the diluted samples were deposited onto LazWell96 plates and evaporated to complete dryness before analysis by LDTD-MS/MS.

The mass spectrometer is operated in positive and negative ionization mode. A flow rate of 6 L/min with air as a carrier gas and a ramp of 6 seconds to 85% laser power with a 1 second hold are used on the LDTD system.

Results: Three-point screening curves (Cut-off, 2 x Cut-off and 5 x Cut-off) and two QCs (QC-0.5X: 0.5 x Cut-off and QC-2X: 2 X Cut-off) were prepared in negative human urine and used to validate the method. The peak area ratio of targeted analyte against the internal standard (IS) ratio was used to normalize the signal. Replicate extractions are deposited on a LazWell plate and dried before analysis.

For the inter-run precision/accuracy experiment, each fortified sample set is analyzed in triplicate on six different runs. For the inter-run, linear calibration curves were performed then accuracy and precision of back calculated concentration were evaluated for each level. Accuracy, %Bias values between -2.4 and 5.7 were obtained and the precision results were lower than 12.1% CV.

For each run, a linear curve through zero with cut-off standards were used to evaluate the QC standards. %CV lower than 17.4% were obtained for the cut-off standard. All QC-0.5X were detected as negative and QC-2X detected as positive.

For the matrix specificity test, 10 different blank matrices were analyzed as unknown sample. All matrices were 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 for 3 day) and dry stability (extracts on LazWell plate for 60 minutes at room temperature) were evaluated. After the given stability time, standards and QCs were analyzed. The precision obtained for cut-off standard ranged between 0.2 and 8.9%CV.

Multi-matrix validations were performed. Real urine was collected from 20 volunteers. Samples were screened using LUXON-MS/MS method and analyzed using LC-MS/MS method. The method sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and accuracy were verified. Finally, a Kappa evaluation is done to verify the interobserver agreement. Kappa scores range from 0 to 1, with 1 indicating perfect agreement and 0 indicating no agreement. A perfect agreement was reached for all drugs.

Discussion: LDTD combined to a Sciex Q-Trap 5500 mass spectrometer system allows ultra-fast (8 seconds per sample) screening of polar drugs in human urine using an automated sample dilution preparation method.

POSTER

P012

Desalkylgidazepam: the new kid on the block in British Columbia

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Abstract

Introduction: Desallkylgidazepam is the latest designer benzodiazepine detected in British Columbia. Desalkylgidazepam is the major metabolite of gidazepam, an approved medication for the treatment of anxiety, alcohol withdrawals and migraines in Ukraine and Russia. Gidazepam and its metabolites activate the gamma-aminobutyric acid type A (GABA A) receptor and the translocator protein (TSPO) and are described as GABA A partial agonists, with its active metabolite desalkylgidazepam showing a 600 times increase in affinity compared to its parent drug. There have been no pharmacokinetic or pharmacodynamic studies published using desalkylgidazepam itself. Desalkylgidazepam was first detected in illicit drugs in Canada in April 2022 by FTIR and confirmed by Health Canada Drug Analysis Service in the same month. Both Erowid's anonymous drug analysis program (USA) and Toronto's drug checking program identified desalkylgidazepam in illicit substances. The CFSRE published a monograph in December 2022. We first detected the drug in post-mortem samples in April 2022, with an average of 10-20 detections per month since then.

This study focuses on the detection, confirmation, and quantitation of desalkylgidazepam in post-mortem blood samples collected and analysed in 2022 in British Columbia, Canada. The co-occurrence of benzodiazepines with opioids in post-mortem samples is also discussed.

Objectives: To quantify desalkylgidazepam concentrations in post-mortem samples and characterize co-occurrences of other drugs that are detected in desalkylgidazepam-positive cases.

Methods: Postmortem samples obtained from cases of suspected illicit drug toxicity were screened by LC/HRMS as part of routine post-mortem screening. Data files were retrospectively analyzed for the presence of desalkylgidazepam, which was confirmed and quantitated by standard addition in whole blood. Samples were extracted by protein precipitation with ice-cold acetonitrile. Diazepam-d^5 was used as internal standard. After centrifugation, the extracts were transferred to an HPLC vial and injected on an Agilent 1290 LC system coupled with an Agilent 6470 triple quadrupole mass spectrometer (Agilent Technologies, Santa Clara, CA, USA). Linearity was evaluated (1-100 ng/mL) as well as the limit of detection (LOD), recovery, matrix effects, carryover and interference from analyte or internal standard and commonly encountered drugs, including but not limited to opioids, over-the-counter medications, benzodiazepines, stimulants, Z-drugs, anti-depressants, and anti-psychotics (n=177). Controls were also prepared at two different concentrations (15 and 75 ng/mL) in blank blood.

Results:The standard addition method was validated succcessfully with a limit of detection of 1 ng/mL, acceptable linearity (r2>0.99), no interference from endogenous or exogenous drugs, acceptable recovery (96-105%) and matrix effects (16%). Desalkylgidazepam was detected in 74 cases and quantitated in 63 postmortem cases in 2022 in British Columbia. The other nine cases could not be quantitated due to insufficient sample volumes. 77% of decedents were male, with an average age of 45 ± 15 years (median: 46 years). Desalkylgidazepam concentrations ranged from 3.7 to 221 ng/mL, with a mean (± SD) desalkylgidazepam concentration of 42 ± 44 ng/mL (median: 24.5 ng/mL) in 2022. Desalkylgidazepam was commonly observed with other benzodiazepines (bromazolam (N=29), etizolam (N=10)), opioids (fentanyl (N=61), carfentanil (N=1) and stimulants (cocaine (N=10)), methamphetamine (N=32))) or ethanol (N=8).

Discussion: Desalkylgidazepam is the latest designer benzodiazepine that could become a public health issue, as its detection in medico-legal death investigations remains constant over time. In this study, desalkylgidazepam was combined with fentanyl in the majority of cases (97%). Combinations of multiple benzodiazepines were also observed in 54% of the cases, with bromazolam and etizolam being individually the most common combination with desalkylgidazepam. Co-occurrence of benzodiazepines with other central nervous system depressants may increase drug overdose risks and lead to more deaths. Laboratories should consider adding desalkylgidazepam to their testing scopes due to its increasing prevalence in post-mortem investigations.

POSTER

P013

An Unusual Suicide by Benzonatate Overdose: A Case Report Utilizing Method of Standard Addition to Confirm Benzonatate Toxicity

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Abstract

Introduction: Benzonatate is a non-narcotic oral antitussive used to treat severe coughs that is only available by prescription. It is a potent sodium channel inhibitor with a narrow therapeutic index that can lead to fatal arrythmias above therapeutic doses. Benzonatate's structural moiety is similar to other polyglycol ester local anesthetics, like procaine, with a variable polymer tail making it difficult to detect by mass spectrometry. Additionally, it is rapidly hydrolyzed into 4-(butylamnio)benzoic acid (BABA), its major metabolite, and other polyethylene glycol monomethyl ethers. Here we report a suicide by benzonatate overdose in a 19-year-old female found unresponsive, collapsed on the bathroom floor, with an empty bottle of benzonatate nearby. The decedent had a history of intentional medication overdoses.

Objective: Since benzonatate and its major metabolite are not routinely detected in our toxicological casework, we aimed to quantitate BABA by method of standard addition using LC-QTOF-MS to confirm a suspected case of benzonatate toxicity.

Method: Antemortem and postmortem specimens analyzed were collected by Dell Children's Medical Center and at autopsy at the Travis County Medical Examiner in Austin, TX, respectively. Specimens were screened for volatiles utilizing HS-GC-FID and drugs of abuse by ELISA. Qualitative drug screens were performed with a validated LC-QTOF-MS method operated in positive ionization mode for the identification of 300+ drugs following a simple protein precipitation. Data were acquired using information dependent acquisition from 100-510Da (MS/MS: 25-510Da, with ramped collision energy 35±15).

Due to benzonatate's unusual (largely unknown) pharmacokinetics, variable size, reference standard availability, and relative rarity of fatal intoxications reported in the literature and from our laboratory, toxicological analysis was challenging and required the development of an assay to confirm benzonatate toxicity via metabolite identification. LC-QTOF-MS analysis of a BABA reference standard was performed using the previously described conditions. Once it was established that the case likely contained notable amounts of BABA, standard addition assessments were performed to determine concentrations in antemortem plasma and blood. Four aliquots of case sample were evaluated with increasing concentrations of BABA added to antemortem plasma and blood: one with no up-spike, and a 500, 1000, and 2000ng/mL up-spike. Based on estimated BABA concentrations, sample dilutions were performed at 40x with drug-free blank blood. Area ratios at each concentration were plotted to determine the x-intercept/BABA concentration in the antemortem plasma and blood specimens.

Results: Autopsy findings demonstrated a normally developed, obese adult female with cerebral and pulmonary edema. Routine toxicology testing identified dextromethorphan (88ng/mL) and chlorpheniramine in antemortem blood. Based on standard addition assessments, BABA concentrations in antemortem plasma (collected 20h before death) and blood (collected 13h before death) were 19 and 6.9mg/L, respectively. Given the sudden nature of the cardiovascular collapse (per medical records), and in the context of elevated benzonatate metabolite concentrations, a fatal arrythmia induced by the toxic effects of benzonatate was determined to be the cause of death. Through autopsy and investigative findings, the manner of death was ruled a suicide.

Discussion: Generally, benzonatate is considered safe under prescribed conditions, and reports of toxicity and fatality are uncommon. However, due to its rapid hydrolysis in the blood and likely low surveillance of BABA by laboratories, toxic episodes may be underreported. If not for the circumstances surrounding this case, benzonatate would have gone undetected. Benzonatate metabolite concentrations may be useful in cases where decedents have access to benzonatate, and no other cause of death is identified. Considering its resemblance to candy and increased prescribing rates among pediatric patients, a higher index of suspicion in pediatric deaths is warranted as a single dose may be lethal in small children.

POSTER

P014

PFAS in Me: Which Ones and How Much?

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Abstract

Bioaccumulation of per- and Polyfluorinated Substances (PFAS) in the human body resulting from environmental exposure is a growing public health concern. Recent studies have linked PFAS exposure to adverse health outcomes including childhood health complications, reduction in kidney functions, thyroid disease, hormone suppression, decreased fertility, increased cholesterol levels, and diabetes, among others. Given the prevalence and ubiquitous nature of PFAS in the environment and everyday consumer products (including our drinking water supply), there is a critical need to develop quantitative tools capable of accurately and precisely detecting low levels of PFAS in biological fluids in order to understand the impact on the human body.

In this study, we combined low-volume blood sampling with the SCIEX QTRAP 7500 system for the analysis of trace levels of PFAS. We present here a quantitative workflow capable of accurately quantifying sub-ng/mL levels of 42 PFAS compounds. The analysis was performed on the author and the results of the analysis are shared to demonstrate what PFAS exposure looks like in a typical American.

Methods: A finger-prick was used to draw capillary blood. The four PFAS-free Mitra tips contained approximately 30 µL of blood each and were stored at -20°C until extraction. Absorptive Mitra tips were then removed from the stem and placed in polypropylene vials with isotopically labeled internal standards or IDAs (Isotope Dilution Analytes) and acetonitrile to aid with protein removal. The samples are sonicated and allowed to equilibrate prior to a centrifuge step to condense the precipitated protein for easier removal. The supernatant was removed and the original tube with Mitra was washed with solvent and the centrifuge step was repeated to ensure that PFAS were not absorbed to the vial. The extracts were then combined and solid phase extraction (SPE) was performed. Injection internal standards (ISs, or recovery standards) were added to the SPE extract immediately prior to placing it in a new polypropylene vial for analysis.

These extracts were injected onto a C18 column at 30°C. A secondary column was introduced as a delay column to counteract endogenous interferences from environmental PFAS compounds present in the system. Data were collected using a SCIEX QTRAP 7500 system using electrospray ionization (ESI) in negative mode. The Scheduled MRM Algorithm was used to optimize data sampling across each peak and maximize the dwell times used.

Results: Recoveries ranged from 76% to 112% with an average recovery of 99% across all analytes. The total amount of perfluorooctanoic acid PFOA detected was 0.82 ng/mL, however, only the linear version of PFOA was detected. This value was slightly lower than the median value of 0.9 ng/mL for Americans aged 18-49 according to the United States Environmental Protection Agency. The total amount of perfluorooctanesulfonic acid (PFOS) detected was 1.862 ng/mL, which again is lower than the median value listed by the EPA of 2.6 ng/mL. Finally, perfluorohexanesulfonic acid (PFHxS) was detected at a value of 1.558 ng/mL or 2.7 times higher than the listed median EPA value of 0.6 ng/mL.

Conclusions: Robust and sensitive workflow for the detection of PFAS in blood samples using the SCIEX QTRAP 7500 system was successfully developed. This low-level sampling approach means that at-home testing of these compounds is possible and can help the population understand their PFAS exposure and the implications PFAS may have on their own health. While the concentrations of PFOA and PFOS presented in this study remained under national median values, the high concentration of PFHxS likely was related to past exposure to aqueous film forming foam (AFFF).

POSTER

P015

The 'Spice' of Life: Identifying a Common Source in a Series of MDMB-4en-PINACA Deaths and a Human Performance Case

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Tarrant County ME, Fort Worth, TX, USA

Abstract

Learning Objective: This poster will describe a series of 7 deaths associated with a single source of MDMB-4en-PINACA, occurring over a 3-month period (March-May 2023) at the Tarrant County Medical Examiner's office (TCME) in Fort Worth, Texas. Utilizing case investigation, postmortem examination and forensic toxicology data, the audience will gain an understanding into the factors that helped identify the single source for this fatal product that was involved with these deaths. This poster will also describe an impaired driving case in which MDMB-4en-PINACA and its metabolite were detected. Attendees will gain insight into the mechanism and manner of death resulting from MDMB-4en-PINACA toxicity, as well as the effects of MDMB-4en-PINACA on driving. The attendee will also gain further knowledge into the detection of both MDMB-4en-PINACA and butanoic acid metabolite in postmortem and impaired driving samples.

Discussion: Synthetic cannabinoids are a diverse group of compounds that are often found in various "legal high" products available for purchase in smoke shops and online. MDMB-4en-PINACA is a newer synthetic cannabinoid, with one of the first reports on its metabolism appearing in the literature in 2019.1 First marketed as 'herbal incense' products, the drug's effects are similar to delta-9-tetrahydrocannabinol (THC). In the US, the Drug Enforcement Agency has temporarily placed the drug into Schedule I status on May 4th, 2023, with at least one US state classifying it as Schedule I.2

As continuously evolving illicit compounds, synthetic cannabinoids primarily act as agonists of cannabinoid receptors and these substances pharmacokinetically behave in a manner similar to THC. However, like other illicit substances, their potency and toxicological effects may vary greatly depending on the compound. Toxicity can exhibit a spectrum of effects in a multitude of organ systems. Psychologic effects can range from slurred speech or impaired motor function to psychoses.3,4,5 Severe physiologic effects include hypertension, hyperthermia, tachycardia, seizures, stroke, pulmonary hemorrhage, renal damage, and cardiomyopathy; it has also been hypothesized that deaths are a result of arrythmia. 6, 7,8 In this presentation, we will present pertinent toxicological findings with respect to a series of 7 deaths associated with a single source of MDMB-4en-PINACA.

In order to better determine if a person expired due to a drug toxicity, postmortem toxicology is essential in any case where drug abuse is suspected. The detection of MDMB-4en-PINACA is possible in blood, urine and various other fluids and tissues utilizing a wide array of analytical instrumentation.9,10 In our cases, LC/QTOF was used to detect MDMB-4en-PINACA and/or its butanoic acid metabolite. These compounds did not cross-react with the cannabinoids ELISA kits utilized by the laboratory. Confirmation was performed by LC/MS/MS.

This poster presentation will provide a general understanding of the pathological findings (or lack thereof) in MDMB-4en-PINACA cases in on our series, as well as discuss the toxicological findings and how a collaborative effort was able to determine a source of the drug.

POSTER

P016

Sensitivity improvement for the detection of steroid hormones and endocannabinoids in keratinized matrices

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Abstract

Introduction: Steroid hormones and endocannabinoids are important endogenous regulators of the human stress response. For the measurement of chronic stress, hair and nail analysis has been found to be the method of choice as it enables a long-term and retrospective determination of endogenous stress markers. The aim of this work was to compare an LC-MS/MS method for the analysis of endogenous steroids and endocannabinoids from keratinized matrices on two different mass spectrometers (SCIEX QTRAP 6500+ and SCIEX 7500 system) to determine sensitivity improvements.

Methods: In this study, analyte extraction followed by a supported liquid extraction (SLE) based sample preparation was implemented for hair and nail samples. Hair and nail samples were first washed for 3 minutes with deionized water, followed by a 2-minute wash with acetone. The washed hair and nail samples were dried overnight at room temperate. Nail clippings (20 mg) were weighed into an Eppendorf tube and 3 milling balls (stainless steel, 5 mm diameter) were added. Nail clippings were pulverized for 10 minutes at 30 Hz. Hair segments were manually cut into snippets and 20 mg of hair was weighed into an Eppendorf tube. Then, 1 mL of methanol and 50 uL of the internal standard were added to each tube containing either the hair or pulverized nail samples. The tubes were briefly shaken and placed in a sonicated bath (35 kHz, 600 W) for 4 hours (hair) or 1 hour (nail) at 55°C for extraction. The tubes were centrifuged at 9000 g for 5 minutes and the methanolic extracts were transferred to a column rack for SLE.

A SLE was performed using an automated Biotage Extrahera system (Biotage, Sweden). Sample extracts were automatically loaded onto Isolute SLE+ columns and allowed to absorb for 5 minutes. Analytes were then eluted 2 times with 2.5 mL ethyl acetate with a wait time of 5 minutes between the 2 elution steps. The extracts were dried in a Turbovap solvent evaporator system (Biotage, Sweden) and resuspended in 60 µL of methanol and 140 µL of a reconstitution solution consisting of 0.2mM ammonium formate in 97:3, water/methanol.

Surrogate analytes were used for the quantification of five steroid hormones (13C3-cortisone, 13C3-cortisol, 13C3-androstenedione, 13C3-testosterone, 13C3-progesterone) and four endocannabinoids (anandamide-D4, 2-arachidonylglycerol-D5, oleoylethanolamide-D4, palmitoylethanolamide-D4) in the sub pg/mg range. Chromatographic separation was achieved using a Phenomenex Kinetex XB-C18 column. The total runtime was 20 min and the injection volume was 2 µL. Source parameters and compound-dependent parameters for all compounds and their corresponding internal standards were optimized on each system, including the Q0D dissociation on the SCIEX 7500 system.

Results: To evaluate sensitivity differences between the two instruments, peak areas and signal-to-noise ratios (S/N) of the calibrators were compared between the SCIEX QTRAP 6500+ and SCIEX 7500 system. Average peak area gains ranged from 9.9x to 71x and 3.7x to 42x for compounds extracted from hair and nails, respectively. The average S/N ratio gains ranged from 0.62x to 13.8x and 0.68x to 19.0x for compounds extracted from hair and nails, respectively. The results demonstrate that the use of the SCIEX 7500 system provided significant increases in peak area and S/N ratios for all steroid hormones and endocannabinoids in the hair and nail.

Discussion: The study showed significant signal-to-noise enhancements for the SCIEX 7500 system as compared to the QTRAP 6500+ mass spectrometer. These results demonstrate that the SCIEX 7500 system can routinely and robustly detect very low levels of analytes extracted from challenging biological matrices. The presented workflow provides the sensitivity levels required for the long-term retrospective measurement of endogenous biomarkers in keratinized matrix.

POSTER

P017

Targeted and non-targeted analysis of fentanyl analogs and their potential metabolites using LC-QTOF

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Abstract

Introduction: The Centers for Disease Control and Prevention (CDC) estimates that approximately 150 overdose deaths occur daily due to synthetic opioids, such as fentanyl and the ~1400 fentanyl analogs. Targeted LC-MS/MS are often unsatisfactory for very large analyte panels and may not detect novel, unknown analogs. Therefore, non-targeted approaches are required for comprehensive detection and confident identification. This study used a high-throughput method to perform targeted and non-targeted screening of fentanyl and its analogs in a single injection of urine samples. A unique feature was the use of Molecule Profiler within SCIEX OS to identify novel fentanyl compounds.

Methods: Frozen urine samples were thawed, mixed, allowed to settle and diluted for LC-MS/MS analysis. LC separation was performed using a Phenomenex Luna Omega Polar C18 column. The SCIEX X500B QTOF system was used to acquire TOF MS and TOF MS/MS data with SWATH DIA. MS/MS library searching was performed for compound confirmation. Screening for drug impurities, fentanyl analogs and metabolites was performed using Molecule Profiler. Candidate precursors were surveyed based on the common fentanyl fragments at m/z 188.1434 and 105.0699.

Results: Targeted screening results showed that fentanyl and acetyl norfentanyl were detected in most urine samples at approximately 8–4300 ng/mL and 26–470 ng/mL, respectively, while most other analytes were not observed. In addition, the Molecule Profiler module of SCIEX OS software was used to search the dataset for potential precursors that shared the common fentanyl fragments. The software generated a list of proposed candidates that included fentanyl, fentanyl analogs, metabolites and impurities based on in silico biotransformation mechanisms. For example, an oxidation product of fentanyl with m/z 353.2224 was consistently observed in many urine samples. In addition, several novel fentanyl biomarkers were identified that were not found during library searching.

Discussion: In this study, a nontargeted analysis of urine samples was performed to detect fentanyl and potential analogs. In addition to targeted screening, the Molecule Profiler software was used to identify novel fentanyl biomarkers using common fentanyl fragments.

POSTER

P018

New hydrolysis and analysis for screening SAMHSA Drug Panel in urine: Development of a rapid screening method using LDTD-MS/MS.

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Abstract

Introduction: The US Department of Health and Human Services (via the SAMHSA agency) has established scientific and technical guidelines for federal workplace programs of drug testing in urine.

Objectives: Use a new approach to hydrolyze the glucuronide form of drugs, and develop an automated sample preparation method for a drug panel in urine using a single operation in Laser Diode Thermal Desorption- tandem mass spectrometry (LDTD-MS/MS).

Methods: The drug list and screening cut-offs requested by SAMHSA guidelines were used. Fourteen drugs were added to negative urine, along with Morphine-3-β-D-glucuronide which was used as an enzyme hydrolysis efficiency QC. Samples, standards, and QCs were extracted using Salt Assisted Liquid-Liquid Extraction (SALLE).

The automated extraction process is performed as follows: matrix is mixed with internal standard, dilution solution, and B-One hydrolysis solution. After a 15-minute incubation at room temperature, a SALLE extraction process was performed by adding 225 µL of extraction buffer (NaCl sat: K2HPO4 (1M) / 1:1) and 450 µL of acetonitrile to get a cleaner sample. A volume of 100 µL of the SALLE upper layer were mixed with 65 µL of desorption solution. A volume of 6 µL were deposited onto LazWell96 plates and evaporated to complete dryness before analysis by LDTD-MS/MS.

The mass spectrometer was operated in MRM positive (21 transitions) and negative (2 transitions) ionization mode. A flow rate of 6 L/min with air as a carrier gas and a ramp of 6 seconds to 55% laser power with a 2 second hold were used on the LDTD system.

Results: Three-point screening curves (Cut-off, 2 x Cut-off and 5 x Cut-off) and two QCs (QC-0.5X: 0.5 x Cut-off and QC-2X: 2 x Cut-off) were prepared in negative urine and used to validate the method for the compound list found in SAMHSA guidelines. The peak area against the internal standard (IS) ratio was used to normalize the signal. Replicate extractions were deposited on a LazWell plate and dried before analysis.

For the inter-run precision/accuracy experiment, each fortified sample set was analyzed in triplicate on five different days. For the inter-run accuracy, %Bias values between -6.5 and 4.8 were obtained and the precision results were lower than 11 %CV. All QC-0.5X were detected as negative and QC-2X detected as positive. Ten drug-free matrices were evaluated, all negative, and an LC-MS cross-validation was performed on authentic samples.

For the LDTD-MS/MS analysis, the wet stability (extracted solutions kept at 4°C for 1 day) and dry stability (extracts on LazWell plate for 75 minutes at room temperature) were evaluated. After the given stability time, calibration curves were analyzed. The precision obtained for standards ranged between 0.6 % and 14.2 %CV and their %Bias accuracy ranged between -7.0 % and 11.1 % of the nominal values.

Multi-matrix validations were performed. Twenty authentic samples were screened using LDTD-MS/MS and analyzed with reference LC-MS/MS method. The method sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and accuracy were verified. The results for the Kappa Score parameters were: The method sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and accuracy were verified using the number of true positive, true negative, false positive, and false negative samples. Finally, a Kappa evaluation is done to verify the interobserver agreement. Kappa scores ranged from 0 to 1, with 1 indicating perfect agreement and 0 indicating no agreement. Perfect agreements were reached for each drug of the SAMHSA panel.

Discussion: LDTD combined with a Sciex Q-Trap 5500 mass spectrometer system allows ultra-fast (10 seconds per sample) screening of SAMHSA drug panel in urine using efficient hydrolysis step and SALLE sample preparation methods.

POSTER

P019

Determination of four diamide-insecticides in blood by liquid chromatography-tandem mass spectrometry: Application to a case of cyantraniliprole poisoning

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Abstract

Introduction: Diamide insecticides are a new class of pesticide that act by binding to the ryanodine receptors and cause the insect's muscle to contract excessively. Four types of diamide insecticides are sold and used in Korea: chlorantraniliprole ($C_{18}H_{14}BrCl_2N_5O_2$, 483.2 g/mol), cyantraniliprole ($C_{19}H_{14}BrClN_6O_2$, 473.7 g/mol), cyclaniliprole ($C_{21}H_{17}Br_2Cl_2N_5O_2$, 602.1 g/mol) and flubendiamide ($C_{23}H_{22}F_7IN_2O_4S$, 682.4 g/mol).

Objectives: Based on our search of the literature, there have been no reports of diamide insecticides poisoning cases in humans. In this study, we present a method for qualitative and quantitative analysis of diamide insecticides in human blood, and results of this application to a real poisoning case.

Method: The extraction of four diamide insecticides in blood was performed using solid phase extraction. Atrazinech-d_5 was used as an internal standard (IS). Diamide insecticides and IS were separated using a reversed phase column (50x3.0 mm, 2.6 μm), and column temperature was kept at 40°C with a flow rate of 0.4 mL/min. The mobile phase consisted of 2 mM ammonium formate and 0.2% formic acid in water and acetonitrile. Mass analysis was performed on a QTRAP mass spectrometer in positive electrosapray ionization (ESI) mode with multiple reaction monitoring (MRM). This developed method was validated for linearity, accuracy, precision, recovery and matrix effect.

Results: In the validation study, the acceptable criteria were satisfied. The calibration curve was found to be linear over the range of 1-100 ng/mL for chlorantraniliprole and flubendiamide and 0.1-20 ng/mL for cyantraniliprole and cyclaniliprole. The validated method was applied to determine cyantraniliprole intoxication in a 67-year-old woman who died at home. She was found with sleeping pills and a pesticide bottle, which was labeled as an insecticide containing cyantraniliprole. According to toxicology results and autopsy findings, the cause of death was determined to be poisoning with cyantrailiprole and zolpidem. In this case study, the concentrations of cyantraniliprole and zolpidem in heart blood were 14.8 mg/L and 1.17 mg/L, respectively.

Discussion: This case study is the first report of blood concentration of cyantraniliprole in a postmortem specimen. It is believed that this method will be useful in cases of poisoning deaths of diamide insecticides that may occur in the future.

POSTER

P020

Ensuring Consumer Health: Diagnostic Ion Screening for Illicit Substances and Prescription Drug Detection in Gas Station Supplements

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Abstract

Introduction: Dietary supplements (DSs) encompass a wide range of products, including vitamins, minerals, botanicals, amino acids, and other substances, intended to supplement the diet. In a study conducted by Mishra et al., 2023, from 2017 to March 2020 the usage of DSs among children, adolescents, and adults in the United States was examined. Findings revealed that approximately one-third of children and adolescents used DSs in the past 30 days, with higher usage among females. Among adults, 58.5% reported DS use, with higher usage among females across various demographic groups. While many individuals consume dietary supplements for various health benefits, concerns regarding their safety and regulation have been raised. Ensuring the quality and safety of dietary supplements is a significant challenge due to the sheer number and diversity of products available.

In this study, a collection of dietary supplements was purchased from gas stations and convenience stores and screened for a range of substances, including novel psychoactive substances, prescription medication, and a variety of botanicals. The samples were chosen based on their popularity and were screened against MS/MS spectral libraries. Several non-disclosed compounds were identified, including prescription medication. Diagnostic ion screening was then performed using Molecule Profiler to identify any impurities related to these prescription drugs.

Methods: Fourteen of the most popular dietary supplements were purchased from gas stations and convenience stores. The supplements were homogenized before analysis and 1 g of sample was placed into 10 mL of acetonitrile. The samples were then vortexed for 1 minute before centrifugation for 5 minutes. Following this, the samples were passed through a 0.22 µm PTFE syringe filter and diluted 1:100 using acetonitrile. Some samples did require additional dilution to maintain an acceptable peak shape.

Liquid chromatography was performed using a Shimadzu LC-40 at a flowrate of 0.6 mL/min using a Phenomenex Luna Omega 3 µm Polar C18, 100 x 2.1 mm (Phenomenex Torrance, CA). The injected sample volume was 10 µL. Mobile phases A and B were Optima grade water with 0.1% formic acid and 5 mmol of ammonium formate and Optima grade acetonitrile with 0.1% formic acid, respectively. Mobile phase B was ramped using a linear gradient from 5 to 100% between 3 and 15 minutes. Mobile phase B was then held for 5 minutes at 100%. Samples were then injected into the ZenTOF 7600 system. MS/MS fragmentation was acquired using SWATH DIA analysis using both collision induced dissociation (CID) and electron activated dissociation (EAD) fragmentation methods.

Results: Seven of the 14 samples disclosed having and did contain Kratom (Mitragyna speciosa). One of the most popular supplements contained a compound called tianeptine, which was not disclosed as an ingredient. In addition to a library fit score of 100 and a parent mass error of -0.5 ppm, an analytical standard was purchased allowing for a level 1 confirmation. In addition to these and other library matches, one of the other supplements contained prescription medications Tadalafil and Sildenafil. Diagnostic ion screening was then used, and 18 impurities related to sildenafil were identified. Several of these compounds were found in spectral libraries, however, many were not found in the literature.

Conclusion: Using high resolution mass spectrometry, several compounds that were not disclosed as ingredients in the supplements were discovered. Notably, the presence of tianeptine and prescription medications, like Tadalafil and Sildenafil, raise concerns about the accuracy of labeling and potential health risks associated with these undisclosed substances. The use of diagnostic ion screening allows for a more comprehensive characterization of these samples through the prioritization of potential compounds of concern even when they are not present in spectral libraries.

POSTER

P021

Simultaneous determination of mescaline, mitragynine, 7-hydroxymitragynine and psilocin in urine samples by liquid chromatography-tandem mass spectrometry

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Abstract

Introduction: Peyote (Lophophora williamsii), kratom (Mitragyna speciosa), and magic mushrooms are representative psychedelic plants and mushrooms. They were initially intended and used for medicinal and ceremonial purposes in some regions, but have been used for recreational purposes globally. In addition to the naturally occurring plants, the extracted active ingredients such as mescaline, mitragynine, 7-hydroxymitragynine, psilocybin and psilocin are regulated in South Korea. In recent years, smuggling and using of psychedelic plants and mushrooms have been increased gradually in South Korea.

Objectives: The rapid detection of these psychoactive compounds in biological materials, especially in urine, is critical to proving illegal use. We developed a simultaneous analytical method for mescaline, mitragynine, 7-hydroxymitragynine and psilocin in urine samples.

Methods: A liquid chromatography-tandem mass spectrometry (LC-MS/MS) method with solid-phase extraction (SPE) was developed and validated for the analysis of mescaline, mitragynine, 7-hydroxymitragynine and psilocin in urine samples. A C18 column (2.1 x 100 mm, 1.8 μm) was used for the separation of these compounds. The MS system was operated in positive electrospray ionization (ESI) using scheduled multiple reaction monitoring (MRM) mode.

Results: The validation parameters including linearity, accuracy, precision, matrix effect, and recovery were satisfactory. The chromatographic separation was performed in 12 min. This method was applied to authentic urine samples which were submitted to the National Forensic Service (NFS) for the determination of the administration of these substances.

Discussion: A total of 18 urine samples were analyzed with this method. Psilocin was detected in one case, and mitragynine and its metabolite 7-hydroxymitragynine were detected in 2 cases. We expect this validated method will be adequate for the detection of the controlled compounds of peyote, kratom and magic mushrooms in forensic field.

POSTER

P022

Comparison of Hydrolysis Efficiency and Performance of Four Recombinant β-glucuronidase Enzymes for the Detection of Opioids in Urine Samples by LC-MS/MS

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Abstract

Background/Introduction: The United States has been experiencing a nationwide public health crisis since introducing prescription opioids in the 1990s. A central problem with opioids is their highly addictive nature causing a dose-dependent respiratory depression that may lead to overdoses and deaths. Drug overdose deaths have risen in the past two decades, with opioids being the dominant driver of this epidemic. Given the circumstances, accurate and efficient confirmatory analysis of opioids in urine is essential to monitoring patients' compliance and preventing drug diversion in clinical and forensic settings.

Objectives: The objective of this study was to optimize and evaluate the enzymatic hydrolysis procedure of seven opioid glucuronide metabolites in urine, including morphine-3-glucuronide, morphine-6-glucuronide, codeine-6-glucuronide, hydromorphone-glucuronide, oxymorphone-glucuronide, norbuprenorphine-glucuronide and buprenorphine-glucuronide, using four different recombinant β-glucuronidase enzymes, BG-Turbo® and B-One® (Kura Biotech), and IMCszyme® RT and IMCszyme® E1F.

Methods: To investigate the optimum hydrolysis conditions of the four recombinant β-glucuronidase enzymes, a set of 500 ng/mL glucuronide mixture controls were prepared in 0.1 mL of drug-free urine. Different volumes of enzyme (20-80 µL) or enzyme-buffer mixture (50-200 µL), temperature (from room temperature to 60℃) and incubation times (10-60 min) were evaluated in triplicates. All the samples were extracted by cation exchange solid-phase extraction, and analyzed by liquid chromatography tandem mass spectrometry in positive ion mode. The optimized conditions were applied to 16 authentic samples positive for opioids. The calibration curve ranged from 5 to 500 ng/mL, and the calibrators were prepared in urine containing the free and the glucuronide forms.

Results: The best conditions to hydrolyze a mixture of 7 glucuronide metabolites at 500 ng/mL each in 0.1 mL urine were determined for each enzyme. For room temperature recombinant enzymes B-One and IMCszyme RT, the optimum conditions were 10 min and 200 µL of the enzyme-buffer mixture, and 15 min and 350 µL of the enzyme-buffer mixture (50 µL enzyme and 300 µL buffer), respectively. Both enzymes hydrolyzed >90% of all of the opioids tested. The optimized conditions for BG-Turbo involved an incubation for 20 min at 55°C using 40 µL of enzyme with 40 µL of Kura Instant Buffer. Under those conditions, the enzyme produced >90% hydrolysis efficiency for glucuronides, except morphine-6-glucuronide (65%). The optimal conditions for IMCszyme E1F were incubation for 20 min at 60°C using 80 µL of enzyme with 100 µL of buffer, producing >90% hydrolysis efficiency for all enzymes, except for codeine-6-glucuronide (75%). The most critical parameter for the room temperature recombinant enzymes, B-One and IMCszyme RT, was the volume of enzyme, and for the other 2 enzymes, BG-Turbo and IMCszyme E1F, was the volume of enzyme and the temperature. All of the enzymes performed an efficient hydrolysis in a short incubation time (10-20 min). All of the authentic samples with different pH (3-9) and opioid concentrations (5 to >500 ng/mL) were successfully hydrolyzed using the optimized conditions.

Conclusion/Discussion: Recombinant β-glucuronidase enzymes efficiently hydrolyzed different types of opioids in urine samples. The most efficient ones were B-One and IMCszyme RT recombinant enzymes, which provided a complete hydrolysis of the opioids (>90%), including morphine-6-glucuronide and codeine-6-glucuronide, at room temperature in less than 15 min.

POSTER

P023

Validation of an Analytical Method for Quantitation of Metonitazene and Isotonitazene in Plasma, Blood, Urine, Liver, and Brain and Application to Authentic Casework in New York City

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Abstract

Introduction: Starting in 2019, an emerging class of novel opioids causing public health concern was benzimidazole opioids, also known as "nitazenes". Two nitazenes, isotonitazene and metonitazene, have been detected in postmortem samples received at the NYC Office of the Chief Medical Examiner.

Objectives: The purpose of this study was to determine the concentrations detected in recent NYC forensic casework, in order to better combat the continued public health issue that is the opioid crisis. A quantitative method was validated for application to pharmacokinetic and casework samples to determine concentrations of novel opioids. Concentrations detected in various matrices and their combination with other drugs of abuse was explored, as well as compilation of demographic and case history data.

Method: A sensitive protein precipitation extraction procedure was developed and validated for metonitazene and isotonitazene using 50 µL of blood, serum, liver, brain, and urine utilizing liquid chromatography tandem mass spectrometry (LC/MS/MS). Cases previously detected qualitatively for these two analytes were selected for testing quantitatively using the validated method.

Results: The limit of detection for both analytes in all matrices was 0.10 ng/mL, except for urine and brain which was 1 ng/mL. The developed method encompassed multiple matrix types, however, many of these were unavailable to be tested in the authentic cases. As a result of the COVID-19 pandemic, autopsy protocols changed to decrease the number of samples provided to the laboratory. Therefore, for most of these cases, only paired blood and urine were available. The method was applied to authentic casework in which isotonitazene was detected in 10 cases between 2021 and 2022, with femoral blood concentrations ranging from 0.11 to 12 ng/mL. Of the isotonitazene positive blood samples that had urine available for testing (n=7), only two were positive, and one was < 1.0 ng/mL. Only one case had liver collected. The liver was poor quality and both the fluid in the specimen cup and a fresh liver tissue homogenate (at 1:5 dilution) were negative. Metonitazene was detected in four cases in 2022. In one of the cases, metonitazene was detected in urine only, with a concentration of 30 ng/mL. The metonitazene blood concentrations were 0.10, 0.49 and 1.5 ng/mL in femoral blood. The two other urine cases tested positive for metonitazene at 1.0 and 14 ng/mL. All but one nitazene case had the presence of fentanyl and/or fentanyl analogs, and the next most commonly encountered analyte in these cases was xylazine. Designer and traditional benzodiazepines, cocaine, methamphetamine, and other opioids were also detected in these samples. The manner of death was accidental for all cases with a detected nitazene. All but one case was attributed to mixed drug intoxications which included the nitazene. None of the cases reported the identified nitazene compound as the sole intoxicant responsible for death.

Discussion/Conclusion: The method developed used microsample volumes to be applied to animal studies for elucidation of pharmacokinetic properties of these analytes and can also be used for other low volume applications. This method utilized is the lowest reported sample volume to date, 50 µL. The opioid crisis continues to tear apart communities throughout the world, including New York City. Continued research and communication regarding these drugs helps to fight this crisis.

POSTER

P024

Head(space) to Head(space): Volatile Analysis by Gas Chromatography Mass Spectrometry/ Flame Ionization Detector (GC-MS/FID) and Gas Chromatography Dual FID

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Abstract

Introduction: Historically the method used for volatile analysis in toxicology laboratories has been Headspace- Gas Chromatography with Dual Column and Dual Flame Ionization Detector (HS-GC-FID). The Headspace-Gas Chromatography- Mass Spectrometry (HS-GC-MS) improves specificity compared to the non-specific carbon-based FID, minimizing or eliminating interferences of the analytes of interest. HS-GC-MS has not been not widely adopted due to the expense and effort in switching instruments and validating new methods.

Objective: To evaluate the performance of the HS-GC dual column and dual FID against the single column HS-GC-MS for the analysis of volatiles analyzed in by a typical forensic toxicology laboratory- ethanol, methanol, isopropanol, and acetone in blood, serum, and urine.

Method: Analytical methods for the determination of ethanol, methanol, isopropanol, and acetone were developed and validated according to ASB Standard 036 for two columns, TG-ALC 1 and TG-ALC Plus II. A TriPlus 500TM Headspace Autosampler equipped with two Instant-Connect FIDs and an ISQ 7000TM GC-MS was used for the analysis. Propanol and t-butanol were used as the internal standards in each column, and propanol was used for quantitation. Seven point calibration curves (100-3,000 mg/L of each volatile) were prepared in deionized water, and controls were prepared in blank whole blood (0. 300, 1,800, 3,200 mg/L of each volatile). Calibrators, controls and samples (100 µL) and ISTD (1 mL) were mixed in 20 mL HS vials. Lower limit of quantitation (LLQ) and Lower limit of detection (LLD) were administratively set at 100 mg/L. Vial incubation time was optimized to 5 min at 80 ºC. Twenty-nine volatile solvents were evaluated as possible interferences and 10 blank blood matrix samples from 10 different sources were analyzed. Sixty-nine blood, serum, and urine clinical samples were analyzed using both columns on the GC-FID and GC-MS.

Results: The method was linear for ethanol, methanol, isopropanol, and acetone. Bias and within-run precision for the GC-MS using both the TG-ALC 1 and TG-ALC Plus II columns was < 15%. Bias for the GC-FID on both columns was < 10% at all concentrations. Between run precision (%CV) for the GC-FID and GC/MS was <10% for both columns. The LLQ and LLD had a bias -11 to 13% and precision was 3 to 12%. Chloroethane was determined to coelute with ethanol on TG-ALC 1 and was resolved on the TG-ALC Plus II GC-FID. The mass spectrometer detector was able to separate the coeluting ethanol and chloroethane peaks by mass. For the clinical samples analyzed, 14 specimens were positive for ethanol. When comparing ethanol concentrations calculated by GC-MS and GC-FID, the GC-MS with the TG-ALC Plus II were determined to have < 25% different. Ethanol concentrations of the GC-MS and GC-FID using the TG-ALC 1 were determined to have < 10% different. Methanol and isopropanol were below the limit of quantitation for all samples. Acetone concentrations of the GC-MS were within 30% of the GC-FID results.

Discussion/Conclusions: The use of dual column HS-GC-FID for blood, serum or urine analysis is important as it allow for the separation/identification of alcohol from potential interferences, notably chloroethane. HS-GC-MS was determined to be an efficient way of analyzing typical volatile using a single column system as the potential interferences could be separation/identification using their mass spectrums. Overall the linearity and precision was better in the dual column HS-GC-FID when compared to either single column HS-GC-MS.

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POSTER

P025

1,1-Difluoroethane (DFE) Prevalence in Kansas

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Abstract

Introduction: 1,1-Difluoroethane (DFE) is a halogenated hydrocarbon (also known as Freon-152a or FC-152a). It is a colorless, odorless gas and is used as a propellant found in many consumer products and electronic cleaners. DFE inhalation can cause rapid CNS depressant effects, altered mental consciousness, hallucinations, and other effects that may impair driving.

Objectives: Determine the prevalence of DFE use in Kansas drivers over the last 7 years and identify demographic trends of DFE use.

Methods: Between March 2016 and May 2023, approximately 13,463 cases were analyzed via Headspace Gas Chromatography with Flame Ionization and Mass Spectrometry Detection to determine the presence of volatiles. The KBI's volatile method includes ethanol (quantitative), methanol, acetone, isopropanol, and DFE. A Limit of Detection (LOD) has been established for DFE at 10 µg/mL.

Results: Of the 13,463 cases analyzed for volatiles during the 7 year time period examined, DFE was identified in 112 (0.8%) cases.

Figure 1 shows the number of positive DFE cases per year and the overall trend. Over the last 7 years, 32 of 105 counties in the state of Kansas had at least one positive DFE case. The highest total for one county was 43 (38%) cases.

Over 70% (81) of cases occurred between 12 pm and 12 am. For the cases where time of incident and collection were both available (38), most samples (23) were collected 1-2 hours post incident.

The majority of DFE users were white males in their 20's. Out of 112 cases, 74% (83) were male. Almost 94% (105) of users were white, followed by 3.5% (4) Hispanic. The 10 year age range of 20-29 had the highest amount of cases (46) followed by 30-39 (38).

Ten cases included a measureable amount of ethanol (0.01 – 0.16 g/100 mL) while 102 tested negative for ethanol at <0.01 g/100mL.

Not all positive DFE cases received additional drug testing. Of the cases that did receive testing for additional drugs (79), 52 had positive results. Of those, approximately 25% (28) were positive for cannabinoids, 9% (10) for benzodiazepines, 8% (9) for stimulants, and 13% (15) were positive for drugs not included in the previous categories. These four categories overlapped in several cases. Based on the data, the trend is that DFE is either used on its own or as part of a poly-drug combination (Figure 2).

Figure 1. Number of positive DFE cases per year (excluding 2023) and overall trend

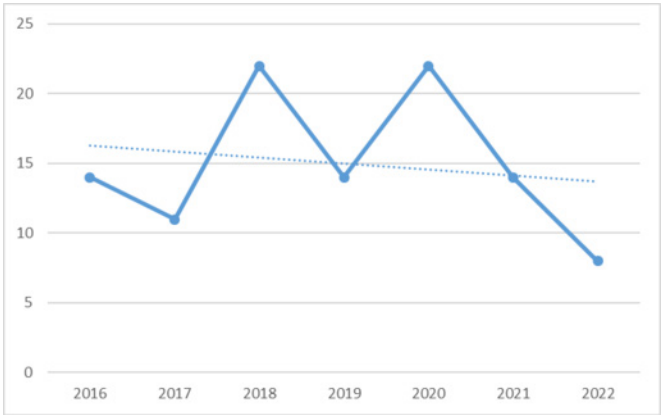

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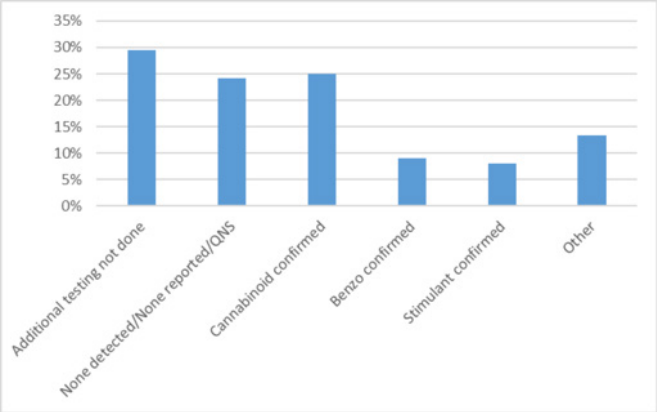
Figure 2. Results of positive DFE cases with other drug testing



Discussion: While DFE cases represent a very small amount of overall casework (0.5% of submissions for volatile and drug testing), it is beneficial to be included in the Toxicology scope of testing. Due to the prevalence of poly-drug use, it would be recommended that all DFE cases receive additional drug testing. Many of the cases reviewed mention a loss of consciousness, crashing into various items (buildings, cars, guardrails, etc), and resulted in fatalities. DFE is a major public health safety issue. Due to the short half-life of DFE, it is important for specimens to be collected in a timely manner to ensure a higher probability of confirming DFE.

P026

Δ8-THC Impact on Non-Regulated Marijuana Confirmation Testing Rates

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Abstract

Introduction: Over the last several years, the use of Δ8-THC products has been documented as a legal alternative to Δ9-THC. The initial widespread use of Δ8-THC began around 2020 and has continued to grow even with legalization of marijuana for recreational and medical purposes. Our laboratory developed what turned into a series of LC-MS/MS methods to adequately separate Δ8-Carboxy-THC and Δ9-Carboxy-THC; maintaining acceptable resolution between analytes required adjustments as each method was challenged with concentrations of Δ8-COOH-THC soaring into the thousands of nanograms per milliliter.

Objectives: The main goals of this study were to determine the prevalence of Δ8-Carboxy-THC in non-regulated workplace drug testing specimens, and reveal the impact of Δ8-Carboxy-THC on the confirmation rate for samples that screened positive by immunoassay.

Methods: In April of 2023, a total of 1,455 urine specimens were initially screened by immunoassay as part of the donor's drug testing panel and then confirmed by LC-MS/MS. The confirmation assay was validated in accordance with National Laboratory Certification Program guidelines for federal drug testing, including interference studies involving 11-Hydroxy-THC, Cannabinol, Cannabidiol, and 126 other drugs. The laboratory collected Δ8-Carboxy-THC and Δ9-Carboxy-THC ions for all specimens with quantitative results for both. The data were correlated with the reason for test, state of collection, and the ultimate reporting result for marijuana and other associated drugs (such as methamphetamine, cocaine, benzodiazepines, etc.). Results for Δ8-Carboxy-THC were only reported to the client when requested as part of the drug testing panel.

Results: All specimen data used was de-identified and detached from client affiliation for this study. Original results for Δ9-Carboxy-THC were reported to the client in accordance with their drug testing policy. Results for Δ8-Carboxy-THC were only reported in two instances. In past years, the Δ9-Carboxy-THC confirmation rate was nearly 100% based on screening positivity rates; however, the confirmation rate for one week in April 2023 fell to 83.6%. Specimens containing only Δ8-Carboxy-THC accounted for 10.3% of the "nonconfirming" samples. The remaining 6% included three samples with high levels of Cannabidiol (CBD), one specimen positive for Δ9-THC with no trace of the Δ9-Carboxy-THC metabolite, and seven samples that were negative for both metabolites, which may have contained other drugs known to cause false-positive immunoassay results (e.g. Protonix, Efavirenz) or alternative cannabinoids.

Overall, 31.2% of the confirmed samples had Δ8-Carboxy-THC concentrations greater than the reporting cutoff of 15 ng/mL, with the highest levels exceeding 19,000 ng/mL. When evaluating by reason for test, collections for Pre-Employment had positive Δ8-Carboxy-THC greater than the reporting cutoff in 48.3% of the samples. Random urine collections were positive for Δ8-Carboxy-THC at a rate of 29.8%.

Discussion: The data obtained in this April 2023 study are similar to a previous study in 2022; however, the amount of Δ8-Carboxy-THC appears to be increasing, as multiple samples had concentrations in the thousands of ng/mL. Due to similar psychological effects of Δ8-THC compared to Δ9-THC, impairment is substantial, a threat to public safety, and should be addressed in public and corporate policy.

Keywords: Δ8-THC, Confirmation, Urine, Non-Regulated

Disclosure

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

POSTER

P027

Separation of 11-Hydroxy-THC Metabolites and Quantitation of 18 Total Cannabinoids in Whole Blood by UHPLC-MS/MS

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Abstract

Introduction: Despite changes in legal status of medical and recreational Δ9-Tetrahydrocannabinol (Δ9THC), employer restrictions and regional accessibility continue to reinforce the popularity of Δ8THC among cannabis users. Toxicology testing for the inactive metabolites Δ9- and Δ8-Carboxy-THC has become more routine, but in many cases the determination of the psychoactive metabolites, 11-Hydroxy-Δ9-THC and 11-Hydroxy-Δ8-THC, is of higher importance. The method developed by our laboratory provides a detailed analysis of whole blood specimens, separating Δ9- and Δ8- 11-Hydroxy-THC metabolites, and quantitatively evaluating 18 cannabinoids at concentrations from 0.500200 ng/mL.

Objectives: Develop an analytical method for the extraction, detection, and quantitation of (-)-Δ9-THC, (-)-Δ8-THC, 11-Hydroxy-Δ9THC, 11-Hydroxy-Δ8-THC, Δ9Carboxy-THC, Δ8-Carboxy-THC, Δ9Tetrahydrocannabivarin, Δ8-Tetrahydrocannabivarin, Δ9-Carboxy-Tetrahydrocannabivarin, Δ8Carboxy-Tetrahydrocannabivarin, Cannabidiol, 7-Carboxy-Cannabidiol, 7-Hydroxy-Cannabidiol, Cannabidiolic Acid, Cannabinol, Cannabigerol, Cannabicyclol, and Cannabichromene in whole blood by LC-MS/MS for a controlled dosing research study.

Methods: Whole blood standard solutions spiked with 18 cannabinoids at known concentrations were analyzed to establish linearity, investigate assay interference, and evaluate matrix effects. Samples were prepared by mixing a 0.200 mL aliquot of whole blood specimen with internal standard solution and 0.1M Sodium Phosphate, adding cold acetonitrile, then centrifuging and decanting the supernatant from the blood protein pellet. A liquid-liquid extraction was performed using the supernatant and 9:1 Hexanes: Ethyl Acetate; the organic components were subsequently dried and reconstituted with 0.1% Formic Acid in 50:50 DI H2O: Methanol. Separation was performed by a Shimadzu Nexera LC40D X3 HPLC system utilizing two Waters™ CORTECS C18+ columns arranged consecutively, with 0.1% Acetic Acid in water and 0.1% Acetic Acid in Acetonitrile mobile phases at a flow rate of 0.7 mL/minute. MS-MS analysis was conducted by a Sciex API7500 tandem mass spectrometer using electrospray ionization in both positive and negative MRM modes.

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Positive Ionization:				
Analyte	Transitions			RT (mins)
	Precursor	Quantifier	Qualifier	
Δ9-THCV	287.2	165.1	123.0	19.90
Δ8-THCV	287.2	165.1	123.0	20.16
CBD	315.2	193.1	135.0	20.31
CBN	311.2	208.0	223.0	22.24
(-)-Δ9-THC	315.2	193.1	123.0	23.66
(-)-Δ8-THC	315.2	193.1	123.0	24.08
Negative Ionization:				
7-COOH-CBD	343.1	297.1	231.1	5.17
Δ8-COOH-THCV	315.2	271.1	163.0	5.51
7-OH-CBD	329.2	299.2	268.1	5.66
Δ9-COOH-THCV	315.2	271.1	163.0	5.92
11-OH-Δ9-THC	329.2	173.0	268.1	13.48
11-OH-Δ8-THC	329.2	173.0	268.1	13.91
Δ8-COOH-THC	343.1	245.1	191.1	14.50
Δ9-COOH-THC	343.1	299.1	245.1	15.54
CBG	315.0	191.1	136.0	20.17
CBDA	357.2	245.1	179.0	20.74
CBL	313.2	191.0	203.0	24.66
CBC	313.2	191.0	203.0	25.17

Results: : Accuracy and precision of 3 replicates at 14 concentrations from 250 pg/mL to 200 ng/mL were used to determine assay linear range around a 5.0 ng/mL single-point calibrator. Linearity was established from 0.50 ng/mL to 100 ng/mL for Δ9-COOH-THCV, Δ8-COOH-THCV, and 7-OH-CBD, and from 0.5 ng/mL to 200 ng/mL for all other analytes. No carryover was observed at the highest concentrations. Replicates at each concentration were within ±20% of target for all analytes, with the exception of Δ9COOH-THCV, Δ8-COOH-THCV, and CBL, which had no labeled internal standard; replicates for these analytes were within ±25% of target. CV was <10% for all analytes except CBL, which was <20%. Matrix effect was evaluated for 10 different donor samples, and interference was investigated with 54 compounds, including over-the-counter, illicit, and commonly prescribed drugs. Neither study revealed issues with identification, quantitation, or ion suppression.

Discussion: The analytical method effectively separated 11-OH-Δ9-THC and 11-OH-Δ8-THC, and reliably identified and quantitated 18 cannabinoids at pg/mL levels, contributing to the scientific knowledge of cannabinoid metabolism and distribution in whole blood. This method demonstrated selectivity, accuracy, and reproducibility for federally-sponsored research studies.

POSTER

P028

Determination of 18 Cannabinoids in Urine with Separation of 11-OH-THC Metabolites by UHPLC-MS/MS

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Abstract

Introduction: With current legal ambiguity concerning both Δ9-Tetrahydrocannabinol and Δ8-Tetrahydrocannabinol, the importance of complete and specific toxicological analysis for parent drugs and metabolites is paramount. While the significance of testing for Δ9- and Δ8-Carboxy (COOH) THC metabolites cannot be understated, changes in employer drug-testing regulations have emphasized the need for the detection and separation of the psychoactive Hydroxy (OH) metabolites of Δ9- and Δ8-THC. The method developed by our laboratory allows for the quantitative determination of 18 different cannabinoids in urine, including 11-OH-Δ9-THC and 11-OH-Δ8-THC.

Objectives: Develop an analytical method for the extraction, detection, and quantitation of Δ9-THC, Δ8-THC, 11-OH-Δ9-THC, 11-OH-Δ8-THC, Δ9-COOH-THC, Δ8-COOH-THC, Δ9-Tetrahydrocannabivarin, Δ8-Tetrahydrocannabivarin, Δ9-COOH-Tetrahydrocannabivarin, Δ8-COOH-Tetrahydrocannabivarin, Cannabidiol, 7-OH-CBD, 7-COOH-CBD, Cannabidiolic Acid, Cannabinol, Cannabichromene, Cannabigerol, and Cannabicyclol in urine by LC-MS/MS for a controlled dosing research study.

Methods: Human urine fortified with bovine serum albumin was spiked with 18 cannabinoids at known concentrations and analyzed to establish linearity and evaluate assay interference and matrix effects. Sample preparation involved dual hydrolysis of a 0.500 mL urine specimen using BG Turbo βglucuronidase/0.1M sodium phosphate solution followed by the addition of 5N Potassium Hydroxide. Samples were then neutralized with 5N Formic Acid and a liquid-liquid extraction was performed using salt-saturated 0.1M sodium phosphate, Acetonitrile, and 9:1 Hexanes: Ethyl Acetate. The organic components were decanted, dried, and reconstituted with 0.1% Formic Acid in 50:50 DI H2O: Methanol. Analysis was executed by a Shimadzu Nexera LC40D X3 UHPLC equipped with two Waters™ CORTECS C18+ columns coupled to a Sciex API6500 tandem mass spectrometer. The aqueous mobile phase, 0.1% Acetic Acid in water, and organic mobile phase, 0.1% Acetic Acid in Acetonitrile, flowed at 0.7 mL/minute over the 29-minute gradient. MS-MS analysis was conducted using electrospray ionization in both positive and negative MRM modes, with 11-OH-THC metabolites being tested in both ionization modes.

POSTER

Positive Ionization:				
Analyte	Transitions			RT (mins)
	Precursor	Quantifier	Qualifier	
11-OH-Δ9-THC	331.2	193.1	201.1	13.70
11-OH-Δ8-THC	331.2	193.1	201.1	14.09
Δ9-Tetrahydrocannabivarin (Δ9-THCV)	287.3	135.0	123.0	19.25
Δ8-Tetrahydrocannabivarin (Δ8-THCV)	287.3	135.0	123.0	19.50
Cannabidiol (CBD)	315.2	193.2	135.2	19.59
Cannabinol (CBN)	311.1	241.0	208.0	21.31
(-)-Δ9-THC	315.2	193.2	123.0	22.40
(-)-Δ8-THC	315.2	193.1	123.0	22.67
Cannabicyclol (CBL)	315.2	235.1	193.0	23.37
Cannabicyclol (CBC)	315.2	193.1	123.1	23.78
Negative Ionization:				
7-COOH-CBD	343.2	297.3	179.0	5.35
Δ8-COOH-THCV	315.2	163.1	217.1	5.74
7-OH-CBD	329.2	268.1	179.0	5.83
Δ9-COOH-THCV	315.2	163.1	217.1	6.16
11-OH-Δ9-THC	329.2	268.1	173.0	13.70
11-OH-Δ8-THC	329.2	268.1	173.0	14.65
Δ8-COOH-THC	343.1	245.1	191.2	14.71
Δ9-COOH-THC	343.2	245.2	191.1	15.82
Cannabigerol (CBG)	315.0	191.1	177.2	19.63
Cannabidiolic Acid (CBDA)	357.2	245.1	227.1	20.74

Results: Accuracy and precision of 3 replicates at 13 concentrations from 500 pg/mL to 500 ng/mL were used to determine assay linear range around a 10.0 ng/mL calibrator. LOQ was validated at 0.5 ng/mL for all analytes. ULOL was established at 500 ng/mL for 7-OH-CBD, Δ9-COOH-THC, Δ8-COOH-THC, and 11-OH-THC metabolites in negative mode; 250 ng/mL for CBG and 11-OH-THC metabolites in positive mode; and 100 ng/mL for all other analytes. Replicates were within ±20% of target for all analytes. CV was <10% for all analytes except Δ9-THCV, which was <15%. Matrix effect and interference studies revealed no issues with identification, quantitation, or ion suppression.

Discussion: The analytical method successfully separated 11-OH-Δ9-THC and 11-OH-Δ8-THC and identified and quantitated 18 total cannabinoids in urine, contributing to the scientific knowledge of cannabinoid metabolism and distribution in urine. Analysis in both ionization modes was considered acceptable for 11-OH-THC metabolites. This method demonstrated selectivity, accuracy, and reproducibility for the analysis of donor urine samples involved in federally sponsored controlled-dosing research studies.

POSTER

P029

Column Selectivity Screening for Analysis of a Panel of Negative Mode Illicit Drugs in Urine by LC-MS/MS Using "Dilute and Shoot"

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Abstract

Introduction: Demand for illicit drug analysis has continued to rise over the last decade. A cost-effective solution that can accommodate an expanded list of drugs, is desired. Laboratories are seeking to expand analytical capabilities shifting toward generic method conditions with minimum sample cleanup, to streamline laboratory workflow. 'Dilute and Shoot' (DnS) is a popular sample preparation approach due to its simplicity and low cost. The goal for 'DnS' is to reduce endogenous impurity components by diluting a sample such as urine, to overcome matrix dependent challenges downstream during analysis. However, success of this approach depends on proper integration of the sample preparation procedure to an analytical method that leverages sensitive MS detection capability. In this communication we have targeted panel of eight analytes: ethyl glucuronide (ETG), ethyl sulfate (ETS), gamma hydroxybutyric acid (GHB) and five barbiturates (Phenobarbital, Butalbital, Amobarbital, pentobarbital, Secobarbital). Nine HPLC columns were screened utilizing a SCIEX 6500+ QTRAP for MS/MS detection. Two columns, a thermally modified Luna 5 µm Omega C18, 50x4.6 mm and a Synergy 2.5 µm Hydro RP, 100x3.0 mm column dimension (from Phenomenex, Torrance CA) exhibited the best performance in terms of retention for the extremely polar ETG, ETS and GHB compounds. However, the Synergy Hydro RP column offered superior analytical capability with better separation of the early eluting, interfering peaks and target analytes in 'DnS' urine samples.

Objectives: The objective of this work is to develop an efficient and reliable chromatographic method for the separation and identification of the eight Illicit drugs in 'DnS' urine samples.

Methods: Mobile phase A and B containing 0.1% formic acid in water and methanol respectively utilized for gradient elution. For Synergy column, mobile phase B was held at 5% for 1.5 minutes following 95% in 0.5 minutes and hold for 2 minutes. Luna Omega utilized a gradient from 2 to 20% B in 1 minute, then to 90% following a hold for 1.5 minutes. The flow rate employed for Synergy and Luna were 400 µL/min and 800 µL/min, respectively. An Agilent 1290 Infinity series LC system was employed with a SCIEX 6500+ QTRAP mass spectrometer under ESI, negative ionization for MS detection. Certified, drug free 100 µL of spiked (50 ng/mL, 25 ng/mL & 10ug/mL concentration for barbiturates, ETG/ETS and GHB respectively, as per lower limit of detection requirement) human urine, diluted fifty fold with mobile phase A following 10 minutes centrifugation. Collect supernatant for injection.

Results: Luna Omega and Synergy Hydro RP column exhibited adequate retention for the most polar (ETG, ETS and GHB) analytes. However, both columns failed to differentiate between the isobaric amobarbital and pentobarbital under similar mobile phase condition, amenable for ETG and ETS in the panel. Hence, reported together. The unique selectivity of the Synergy column yielded better separation of the target analytes from urinary endogenous interferences, demonstrating improved signal sensitivity for most analytes. Use of scheduled MRM detection further aided in elimination of unwanted isobaric impurities leaving the MS acquisition window cleaner, enabling easy quantitation of target analytes.

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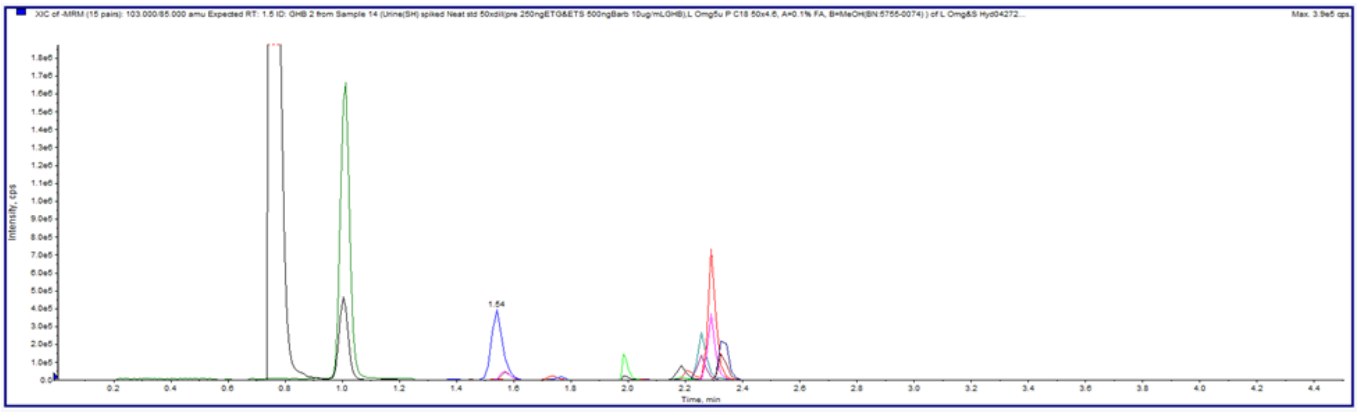
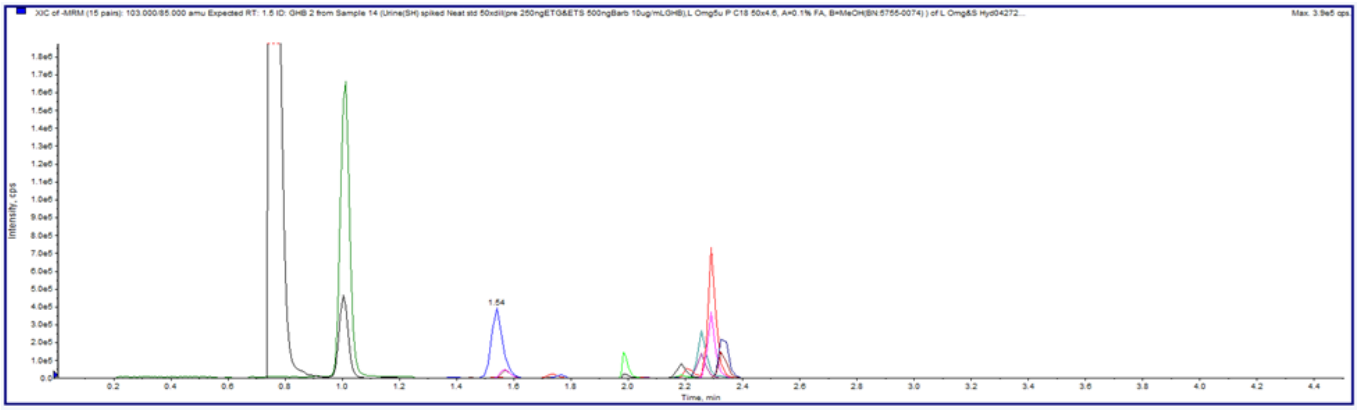
Figure 1. Representative chromatograms for injected 'DnS' samples on respective columns

a) Luna Omega C18



b) Synergi Hydro RP



Discussion: The proper integration of 'DnS' sample prep approach to the developed LC-MS/MS method utilizing a Synergy 2.5 µm Hydro RP, 100x3.0 mm column can simplify laboratory workflow, reducing operating cost for multi-class drug analysis.

POSTER

P030

Testing DUID Casework for all Tier One Drugs: A 18-month Review

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Abstract

Introduction: Following comprehensive ASB/ANSI standard 036 validation, a routine method testing for 127 compounds was employed by the San Francisco Office of the Chief Medical Examiner (SFOCME) Forensic Laboratory Division. The scope of testing for this method meets and exceeds the ASB 120 scope and sensitivity standards as well as the National Safety Council's Alcohol, Drugs and Impairment Division's recommendations for Tier I drug testing in Driving Under the Influence of Drugs and/or Alcohol (DUID) casework.

Objectives: The objective of this review was to compile and present the confirmed drug detections generated via routine testing for 127 drugs of abuse within all DUID casework.

Methods: Following the implementation of this method, the SFOCME Forensic Lab Division began testing all DUID casework in-house, starting July 1, 2021. Data analysis and interpretation was performed on all DUID casework results from July 1, 2021, to December 31, 2022.

Results: The data for the time frame of July 1, 2021, to December 31, 2022, revealed consistency regarding the seasonality of impaired driving within the City and County of San Francisco, the prevalence of various drug classes within the community, the individual drugs being abused, and the commonplace of polysubstance use among the impaired drivers of San Francisco. Alcohol was present in 80% of cases. Among those cases, 89% of drivers had a BAC above 0.08%, with 35% of all cases falling between a BAC of 0.15%-0.21%. Polysubstance use was the most common result with 56% of all cases positive for any combination of alcohol, cannabis, and/or drugs.

Discussion: The interpretation of the data results in some discussion regarding the effect of a broad testing scope on the rate of cases with drivers positive for impairing substances. The rate of positive identifications also warrants a discussion regarding the number of impaired drivers within the community, the effectiveness of law enforcement's ability to recognize impairment or a combination of both. With DUID casework averaging over 400 cases annually for the SFOCME Forensic Laboratory Division, thousands of data points are generated. The ability to collect and curate this data for policymakers is key in providing insights to enable drug harm reduction efforts to prevent traffic fatalities.

POSTER

P031

Prevalence of Xylazine and Fentanyl in Umbilical Cord Specimens from High Risk Populations in the United States

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Abstract

Introduction: Xylazine is a nonopioid, FDA approved veterinary tranquilizer. Human use was rejected by the FDA due to frequency of severe hypotension and central nervous system depression. However, xylazine is now being found in illicit drug supplies, and is combined with drugs like fentanyl to prolong the effects of 'feeling high'. Fentanyl, the synthetic opioid used treat severe or complex pain, has transformed the illegal drug market with its high potency and ease of synthesis.

The combination of xylazine and fentanyl is known as 'Tranq Dope' and has become increasingly popular amongst illicit drug users. In 2022, the DEA laboratory system reported approximately 23% of fentanyl powder and 7% of fentanyl pills to contain xylazine from seized drugs. With 'Tranq Dope' use on the rise, it is important to evaluate the risk of exposure in the neonatal population.

Objectives: The objective of this study was to determine the presence of xylazine in umbilical cord specimens positive for fentanyl and to evaluate the positivity rates in five regions of the United States.

Methods: One-hundred and one previously confirmed fentanyl positive umbilical cord specimens from babies born in the month of April 2023 were analyzed for the presence of xylazine. Roughly 0.5 g of umbilical cord specimen was homogenized with acetonitrile and isotopic internal standard followed by solid-phase extraction with mixed mode cartridges using the Biotage® Extrahera™. The specimens were dried down in a Biotage® TurboVap® evaporator and reconstituted with 0.1% Formic Acid/6% Acetonitrile, then analyzed by LC-MS/MS.

In addition, the demographics of the sample population were evaluated by dividing the US into five regions: West, Southwest, Midwest, Northeast and Southeast. Previously acquired data from the population was also reviewed to determine co-exposure to other drugs of abuse other than fentanyl and xylazine.

Results: Xylazine tested positive in 29 out of 101 positive fentanyl specimens, with concentrations ranging from 0.34 – 1322.54 ng/g (median = 2.13 ng/g). Xylazine positive samples all contained fentanyl and norfentanyl, with median concentrations of 15,832 ng/g and 24,798 ng/g, respectively. In addition to xylazine and fentanyl, 96% of the specimens were positive for co-exposure to 2 or more drugs, including amphetamine, methamphetamine and/or cocaine. Ten specimens had co-exposure to 3 additional drugs, and 4 additional drugs were found in 3 specimens.

The demographic data is shown in Table 1. The Southeast region had the highest positivity rates of the sampled population with fentanyl at 32.3% and fentanyl with xylazine at 55.1%. States with the highest positivity rates in the Southeast region included Kentucky, Tennessee, and Florida. No xylazine positives were found in the West and Southwest regions.

Table 1: Regional positivity rates of Umbilical Cord Specimens

US Regions	Fentanyl (n = 101)	Xylazine & Fentanyl (n = 29)
Southeast	32.30%	55.10%
Midwest	28.40%	37.90%
West	27.40%	0%
Southwest	7.80%	0%
Northeast	3.90%	6.80%

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Discussion: Xylazine was detected in 28% of fentanyl confirmed positive umbilical cord specimens in April 2023, with most specimens confirming for 2 or more additional drugs of abuse. At the time of the study, xylazine was not found in specimens from the West Coast, highlighting regional differences in illicit use.

Street drugs can be a game of Russian roulette for both users and medical professionals. Current medical practices rely on toxicology testing that may not include newly trending drug use like 'Tranq Dope'. These results encourage laboratories in regions with significant prevalence of xylazine to further validate screening and confirmation analysis in newborn testing to enhance the treatment of prenatal exposure.

P032

Simultaneous determination of Δ^8-THCCOOH and Δ^9-THCCOOH in hair by LC-MS/MS

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Abstract

Introduction: Hair analysis has been regarded as an alternative method to urine analysis in forensic and criminal cases. Identification of 11-nor-9-carboxy-tetrahydrocannabinol (Δ^9-THCCOOH) in hair can be an important indicator of cannabis use because it can exclude the possibility of passive cannabis smoke exposure. In recent years, Δ^8-THC has emerged dramatically in seized materials such as e-cigarettes liquids, foods, and smoking tools submitted to National Forensic Service (NFS). In South Korea, Δ^8-THC is also a controlled narcotic. In this study, quantitative determination of Δ^8-THCCOOH and Δ^9-THCCOOH in hair was performed by LC-MS/MS.

Methods: Liquid-liquid extraction (LLE) after alkaline hydrolysis of hair samples, selective column switching liquid chromatography with electrospray ionization (ESI)-MS^3 were used for the determination of Δ^8-THCCOOH and Δ^9-THCCOOH in hair samples. For the column switching system, three columns (pre column, trap column and analytical column) were used. The internal standard was THCCOOH-D_3. We used ESI-negative-MS^3 transition of ions at m/z 343 to 299 to 245 for quantification of Δ^9-THCCOOH and Δ^8-THCCOOH.

Results: The validation results of selectivity, matrix effect, recovery, linearity, precision, accuracy, and processed sample stability were satisfactory. The limit of detection (LOD) of Δ^8-THCCOOH and Δ^9-THCCOOH was both 0.05 pg/mg. This method was applied to authentic hair samples that were submitted to NFS for the determination of cannabis use. A positive result was quantitated for Δ^8-THCCOOH and Δ^9-THCCOOH in 83 cases.

Discussion: In 16 cases, only Δ^8-THCCOOH was detected, whereas Δ^8-THCCOOH and Δ^9-THCCOOH were detected in 67 hair samples. The range of concentration of Δ^8-THCCOOH and Δ^9-THCCOOH in hair was 0.074~472.55 pg/mg and 0.06~18.80 pg/mg, respectively. This method was successfully applied in the analysis of authentic human hair samples for the determination of cannabis use in forensic field.

POSTER

P033

LC-QTOF-MS Method Development and Validation for the Screening of Nitazene Analogs in Whole Blood

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Abstract

Introduction: Since 2019, the emergence of nitazene analogs has been a concern for the forensic community. Nitazenes produce effects similar to those of opioids and fentanyl analogs and are particularly concerning for their potency. Since nitazene compounds are relatively new to the market and quickly change in popularity, traditional screening methods like immunoassay can potentially produce false negative results. Liquid chromatography quadrupole time-of-flight mass spectrometry (LC-QTOF-MS) can be used as a comprehensive screening method where emerging compounds can be added to existing libraries allowing for easy integration into existing methods. When available, LC-QTOF-MS can offer more sensitivity and selectivity over immunoassay as a screening method for novel drugs of abuse.

Objectives: The primary aim of this study was to develop and validate an extraction and LC-QTOF-MS method for the screening of seven commonly encountered nitazene analogs (4'-OH nitazene, 5-methyl etodesnitazene, isotonitazene, metodesnitazene, N-piperidinyl etonitazene, N-pyrrolidino etonitazene, and protonitazene) and two internal standards (isotonitazene 13C6 and metodesnitazene-D4). Notably, this is one of the first methods to include 5-methyl etodesnitazene to the analysis panel.

Methods: A liquid-liquid extraction (LLE) was developed using 500 µL of blood with the addition of 25 µL (final concentration 20 ng/mL) internal standard. Ammonium hydroxide and 10 mM borate buffer (pH 9) was added before extraction solvent (100% chlorobutane). Following solvent addition, samples were rotated and centrifuged prior to the transfer of the organic layer. After drying, samples were reconstituted in 200µL of 90:10 mobile phase A (0.1% formic acid with 5 mM ammonium formate in water) and mobile phase B (0.1% formic acid in acetonitrile).

An Agilent 1290 Infinity Liquid Chromatograph coupled to an Agilent 6530 Accurate Mass QTOF was used for analysis. A Poroshell 120 EC-C18 column (100 x 2.1 mm x 2.7 µm) with matching guard was used for chromatographic separation. Agilent MassHunter Qualitative analysis with Agilent Personal Compound Database Library (PCDL) was used for data analysis. Peak areas were used for limit of detection and matrix effects calculations.

Results: The developed method was successfully able to chromatographically separate all analytes of interest with baseline resolution, including isomers isotonitazene and protonitazene. The gradient used was as follows: 10% B start → 90% B over 5 minutes → 2 minute hold → 3 minute re-equilibration for a total run time of 9 minutes. Limit of detection (n=6) ranged from 1.0-5.0 ng/mL depending on the compound. Ionization suppression/enhancement (n=10) ranged from -46% to 53% at the low concentration (5 ng/mL) and -57% to -24% at the high concentration (25 ng/mL). Matrix interferences were observed in the metodesnitazene window but did not affect peak integration and the matrix effects observed did not affect detection or reproducibility.

Discussion: As nitazene analogs become increasingly prominent, forensic laboratories need novel drug screening methods that can adapt to frequently changing compound prevalence. This study produced an efficient screening method using LLE and LC-QTOF-MS for seven commonly encountered nitazene analogs that was validated according to ASB 036. This study is one of the first to validate for 5-methyl etodesnitazene. This method, combined with PCDL processing, allows the easy integration of emerging novel compounds to existing methods and can help reduce the instances of false negatives from analytes with poor cross-reactivity to immunoassay kits.

POSTER

P034

LC/MS/MS analysis of 11-nor-9-carboxy-tetrahydrocannabinol in fingernails

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Abstract

Introduction: Hemp is regulated under the Narcotics Control Act in Korea. Urine and hair samples are mainly used as biological samples to prove cannabis abuse. In general, the main metabolite of cannabis, 11-nor-9-carboxy-tetrahydrocannabinol (Δ9-THCCOOH) among the other cannabis metabolites is analyzed to determine whether cannabis has been abused in order to exclude the possibility of external contamination. Recently, 11-nor-8-tetrahydrocannabinol(Δ8-THC), an isomer of Δ9-THC which is synthesized with cannabidiol is also abused in the form of e-cigarettes, and its metabolite is 11-nor-8-carboxy-tetrahydrocannabinol (Δ8-THCCOOH).

Objectives: Currently, analysis of narcotics using fingernails has been established only for methamphetamines in Korea. Although THCCOOH analysis in urine and hair had been established, but THCCOOH assays in nails has not been established. Fingernails of three people suspected cannabis abuse had been submitted for THCCOOH assay. Therefore we tried to analyze these cannabis metabolites in fingernails.

Methods: Method validation was performed for Δ8-THCCOOH and Δ9-THCCOOH with Sciex 6500 LC/MS/MS to confirm the main metabolite of hemp component in the fingernails. Instrumental condition for THCCOOH analysis is the same as method used for hair analysis, column switching LC–ESI-MS3. Carboxy-tetrahydrocannabinol was extracted by liquid-liquid extraction in digested fingernails with 1N NaOH. ESI-negative-MS transition of ions at m/z 343 to 299 to 245 (343/299/245) and m/z 346 to 302 to 248 (346/302/248) for quantification of THCCOOH and Δ9-THCCOOH-d3 were used, respectively. Calibration curve ranged from 0.1 to 20 pg/mg and R2 was 0.99 or more, and LOD and LOQ of both components were 0.05 pg/mg and 0.1 pg/mg respectively. Accuracy and precision, matrix effect, recovery and process efficiency all showed values within 15% as CV values.

Results: According to this method, the fingernails of three people were analyzed, and as a result, Δ9-THCCOOH in the fingernails of one person was detected and the concentration was 12.29 pg/mg. Fingernails of the others showed negative results. THC was also detected in all related evidences of the positive abuser.

Discussion: Method validation results of THCCOOH analysis in fingernails were not significantly different from those in hair, including LOD and LOQ results. It is sometimes difficult to obtain hair as an evidence to prove cannabis abuse, then fingernails are recommended as a substitute for hair samples.

POSTER

P035

Best Practices: Hydrolysis Protocols Are Not a "One Size Fits All" for Each Drug Class, when using B-One

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Abstract

Introduction: Glucuronidation plays a crucial role in the elimination of many drugs from the body. This metabolic process involves the conjugation of drugs with glucuronic acid, resulting in the formation of more polar compounds that can be easily excreted. Consequently, these conjugated drugs can complicate drug analysis by mass spectrometry for multiple reasons and to help resolve this issue, many laboratories utilize beta-glucuronidase enzymes to cleave the glucuronide conjugates to convert them back to their original, parent form. This approach allows for more accurate detection and quantification of these drugs and metabolites while streamlining the sample preparation workflow. However, it's important to take into consideration when hydrolyzing different drug classes such as opiates/opioids, benzodiazepines, cannabinoids, and antidepressants, utilizing a universal approach may not be the most effective option. The reason for this is that each analyte has a unique interaction with beta-glucuronidase, leading to a customized hydrolysis method that is tailored to the specific class of drugs.

Objective: Our main objective is to emphasize that a one-size-fits-all approach is not always ideal when hydrolyzing different drug classes and analytes, and to raise awareness about the importance of a customized hydrolysis protocol. This study also shows how to apply best practices while evaluating the hydrolysis performance using B-One® (Finden® by Kura Biotech®) recombinant beta-glucuronidase for the various drug classes for use in forensic and clinical toxicology laboratories.

Materials and Methods: Common drug classes and analytes were quantitatively analyzed in different panels for hydrolysis efficiency using the following glucuronide standards fortified in drug-free urine: Benzodiazepines, Naloxone/Buprenorphine, Carboxy-THC, and Amitriptyline. Custom standard mixes which include calibration and internal standards as well as glucuronides for the quality controls standards were from NGX for this study. Quality control standards were prepared at low and high concentrations, 500 ng/mL and 5,000 ng/mL respectively. The hydrolysis method was performed using B-One®, an "all-in-one" recombinant beta-glucuronidase stabilized in its reaction buffer for quick room-temperature hydrolysis. The hydrolysis was followed by a clean-up protocol using XTR™ tips 5 mg HLB (DPX Technologies) and then diluted with DI water for analysis by LC-MS/MS.

Results: A quantitative method was used to determine the concentrations of free drugs for each analyte in quadruplicates and then the recoveries were calculated using a hydrolysis efficiency formula. Results demonstrated good recovery and precision with an optimized hydrolysis method for each drug class.

Discussion and Conclusion: The hydrolysis experiments conducted on various drug classes demonstrate the need for customized hydrolysis parameters for B-One,® including different hydrolysis times and enzyme amounts, by following the best practices suggested here. The addition of DPX XTR™ tips and NGX custom standard mixtures provides a streamlined protocol for ease of use as well.

POSTER

P036

Urine Drug Surveillance in Philadelphia, PA, with Emphasis on Xylazine and its Metabolites

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Abstract

Introduction: Over the past decade, the heroin supply in the United States has diminished as the fentanyl supply has greatly increased along with the presence of adulterating agents, most notably xylazine. Xylazine is a veterinary sedative not intended for human use; however, currently more than 90% of recreational opioid samples (primarily fentanyl) collected from Philadelphia, PA, also contain xylazine. The Center for Forensic Science Research and Education recently partnered with the Philadelphia Department of Public Health to pilot expanded analysis of urine samples collected in the Police Assisted Diversion program. This program engages individuals who encounter law enforcement for non-violent low-level offenses to provide referrals to behavioral health services, social services, public benefits, and medical services. Clients who received an assessment for behavioral health services provided urine samples.

Objectives: This study involved three main objectives: 1) qualitative analysis of urine samples by liquid chromatography quadrupole time-of-flight mass spectrometry (LC-QTOF-MS) to determine drug prevalence, 2) comparison of the test results to BTNX™ Rapid Response Xylazine Test Strips (XTS) to determine effectiveness, and 3) characterization of xylazine and its known metabolites, 4-hydroxy xylazine and 2,6-xylidine, to determine the appropriate urine biomarker.

Methods: From May through October 2022, 412 urine samples were collected and sequestered for testing. Samples were prepared unhydrolyzed for LC-QTOF-MS analysis using two extraction procedures – a basic liquid-liquid extraction and an acidic liquid-liquid extraction. Instrumental analyses were completed using two validated LC-QTOF-MS platforms: a SCIEX TripleTOF™ 5600+ and a SCIEX X500R. Datafiles were processed against an internal library database of more than 1,000 analytes. Standard reference materials for xylazine and its metabolites were acquired in addition to that database. The xylazine LOD was <5 ng/mL.

Testing with the BTNX Rapid Response™ XTS (manufacturer listed cutoff: 1,000 ng/mL) was conducted following the manufacturer's guidelines. A positive control (2,000 ng/mL) and negative control (drug-free urine) were prepared alongside the authentic urine samples. Results were tabulated and compared to prior qualitative LC-QTOF-MS results for further data analysis.

Results: Based on the LC-QTOF-MS results, approximately 90% of all urine samples screened positive for fentanyl, with fluorofentanyl (65%) being the primary fentanyl analog detected. Methamphetamine, cocaine, and/or benzoylecgonine were present in approximately 70% of samples. Approximately 25% screened positive for carboxy-THC. Novel psychoactive substances (NPS) were not frequently detected, but 8-aminoclonazolam (4%) was observed. The LC-QTOF-MS results showed 88% of the urine samples screened positive for xylazine, and of those positive samples, 53% also contained 4-hydroxy xylazine. Only 13% of the positive samples also contained 2,6-xylidine, while the remaining 46% of positive samples did not contain either metabolite. All urine samples contained parent xylazine.

Comparison of XTS urinalysis results with LC-QTOF-MS results showed acceptable performance. The sensitivity was calculated to be 87%, the specificity was 80%, and the accuracy was 86%. The qualitative LC-QTOF-MS results were categorized as positive/negative and were not evaluated quantitatively against the cutoff; therefore, a higher number of false negatives (n=48) may be reported due to increased sensitivity of the LC-QTOF-MS assay vs. the XTS. Additionally, the sample population was highly saturated with xylazine positivity leading to a higher proportion of true positives (n=314) compared to true negatives (n=40).

Discussion: Our urine surveillance showed that Philadelphia, PA, is experiencing high positivity for xylazine, with fentanyl being the primary drug in urine samples from this population. Methamphetamine and cocaine were also commonly detected. Metabolite characterization showed that 4-hydroxy xylazine was the primary metabolite; however, xylazine remains the appropriate biomarker for toxicology testing. Overall, the performance of the XTS was adequate for detecting xylazine in the urine samples analyzed when compared to comprehensive LC-QTOF-MS drug screening.

POSTER

P037

The Evolving Landscape of THC Drug Testing, Delta-8 vs. Delta-9

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Abstract

Introduction: For THC drug testing, the carboxy metabolite is historically the analyte used to determine cannabis usage. This compound has a long half-life and can be detected in urine or blood for several weeks in heavy consumers. This can pose a challenge when determining if a user is intoxicated at the time of testing. Today, labs are interested in the addition of the hydroxy metabolite, the intermediate between THC and the carboxy metabolite. The intermediate is short lived, but it is useful in the determination of chronic usage and when determining if a user is under the influence. Delta-8-THC is a common isomer of delta-9-THC that also demonstrates psychoactive effects and is of analytical interest to drug testing laboratories. The chromatographic separation of delta-9-THC and delta-8-THC and their respective metabolites are required due to their shared masses. Several column chemistries were scouted, and a method was developed to separate delta-9-THC and delta-8-THC as well as their carboxy and hydroxy metabolites.

Objectives: Three column chemistries were tested to determine which stationary phase is able to achieve the best resolution of isomers in the shortest amount of time.

Methods: Biphenyl, ARC-18, and FluoroPhenyl stationary phases were tested on a 100 x 2.1 mm column dimension using water and methanol as mobile phases, both modified with 0.1% formic acid.

Results: The Biphenyl stationary phase did not display selectivity for the three pairs of isomers under the scouting conditions tested. The ARC-18 phase showed selectivity for both the delta-9/8-THC isomers and their carboxy metabolites, but not the hydroxy metabolites. When the strength of the solvent is reduced to attempt to resolve the hydroxy metabolites, the THC isomers become excessively retained, and the hydroxy metabolites are still not resolved. The FluoroPhenyl stationary phase shows selectivity for all compounds under initial scouting conditions. The gradient was adjusted in order to achieve near full resolution of the THC isomers but this resulted in a long analytical runtime. It was found that the separation of all compounds was achievable in a 12-minute cycle time by dropping the temperature from 40 °C to 30 °C paired with the use of isocratic conditions. FluoroPhenyl showed selectivity for all three pairs of isomers and a method to fully resolve them was developed on a 100 x 3 mm column.

Discussion: Three column chemistries were investigated in this study for the analysis of delta-8/9-THC and their carboxy and hydroxy metabolites. The FluoroPhenyl column shows great selectivity for the target analytes and can resolve all three pairs of isomers with great resolution and retention with a 12-minute total cycle time.

POSTER

P038

Demographic differences in drug-related deaths

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Abstract

Background: Quantifying drugs detected in post-mortem (PM) blood is essential for determining cause of death. However, the amount of drug which relates to non-fatal toxicity in one individual may relate to fatal toxicity in another due to demographic differences in age, biological sex, and ethnicity. This work aims to identify toxicological differences in drug-related deaths in demographic sub-groups within the United Kingdom (UK) to aid toxicologists, pathologists, and coroners in interpreting toxicology results.

Methods: Data reported to the National Programme on Substance Abuse Deaths (NPSAD) was extracted (between 1997 to 2021) and analysed. Drugs with ≥6 quantifications were included for analysis. Median concentrations and 95th percentiles were calculated and delineated by age, gender, and prescribing status for cases where the drug was detected at PM but not implicated in causing death (PM Only), and those where the drug was detected and implicated (PM & Implicated).

Results: A total of 101 drugs had ≥6 quantifications reported to the NPSAD, with 30 of these drugs having now been analysed including alprazolam, promethazine, methadone, and codeine. Significant variations in drug concentrations were observed between PM Only and PM & implicated cases, and between decedent demographics. For example, in adult male cases where methadone was detected at PM & implicated in causing death, those prescribed methadone had a median concentration of 0.70mg/l (n=982), whereas those not prescribed methadone had a significantly lower median concentration of 0.43mg/l (n=1,844; $p<0.05$). Furthermore, adult Asian males had a significantly higher median concentration of methadone at PM & implicated in causing death (0.62mg/l, n=28) than adult males from Black (0.48mg/l, n=14) backgrounds.

Conclusion: This study demonstrates important toxicological differences in drug overdoses based on age, gender, tolerance, and ethnicity. These demographic factors therefore need considering in the interpretation of toxicology results. While this study highlights toxicological differences among demographic groups, further analysis of additional factors is needed for definitive conclusions.

POSTER

P039

The Emergency of Bromazolam in Jefferson County, Alabama: A Case Series

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Abstract

Introduction: Postmortem toxicology is an ever-changing landscape presenting challenges for both toxicologists and medical examiners. Benzodiazepines are a class of drugs that tend to be transient, with specific drugs changing in popularity every few years. Some such as diazepam, are consistent, but others such as alprazolam and clonazepam ebb and flow depending on prescription trends and street popularity. Novel benzodiazepines such as etizolam are even more transient and vary in casework depending on legal restrictions and black market availability.
Objectives: Bromazolam, a designer benzodiazepine, is the brominated analog of the low dose benzodiazepine alprazolam and is likely more potent. There is a lack of available information in the scientific literature on bromazolam concentrations in fatal and non-fatal cases, however the contribution of benzodiazepines to opioid fatalities is well understood. As bromazolam is a potent benzodiazepine, low concentrations of this novel drug are likely to contribute to CNS depression in opioid overdose cases.
Methods: In June 2022, the first case of bromazolam was detected by gas chromatography-mass spectrometry (Cayman Chemical Company mass spectral search) in the Jefferson County Coroner/Medical Examiner Office (JCCMEO) casework performed at the University of Alabama at Birmingham's Forensic Toxicology Laboratory. Between then and March of 2023, bromazolam was detected in an additional six cases. We present the features of the seven cases in which bromazolam was detected at JCCMEO.
Results: The decedents ranged in age from 20 to 35 years of age (median age of 28.1 years). Most of the decedents were white (n = 5; 71.4%) and male (n = 5; 71.4%). Bromazolam was found in concentrations ranging from <0.025 to 0.108 mg/L with a mean concentration of 0.057 mg/L. In all cases, bromazolam was among a mixture of drugs detected in each case with all of the deaths being related to drug toxicity. Fentanyl was detected in 6 of the 7 decedents in concentrations ranging from <0.0025 to 0.097 mg/L with a mean concentration of 0.028 mg/L. Additional drugs detected were methamphetamine, ethanol, oxycodone, methadone, cocaine, amphetamine, morphine, and diphenhydramine.
Discussion: While all manners of death were ruled as accidental, bromazolam was included in the cause of death statements in six of the seven cases (listed with other illicit substances like fentanyl and methamphetamine). Capturing important emerging drug trends on the death certificate is critical to help inform public health and medical colleagues for preventive measures and treatment in the continued drug epidemic.

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P040

Death by Depressants – a Case Study in Drug and Ethanol Postmortem Distribution

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Abstract

Introduction: A 49-year-old male was found unresponsive in the front seat of his vehicle on the shoulder of the freeway with no indication of a crash or crime. Inside the vehicle, multiple pills were located along with unopened Nighttime sleep aid and Nyquill Severe, emesis, water bottles, and a cup of brownish-yellow liquid. Located just outside the car was more emesis, a 1 L bottle of vodka with under 0.25 L left, and an empty bottle of Tylenol.

The decedent had a reported history of occasional alcohol use, insomnia, and hyperlipidemia. He was prescribed zolpidem, carbamazepine, and gabapentin. He was hospitalized for an accidental zolpidem overdose about 2 years before his death and had no history of suicidal ideation or attempts.

Objectives: This case study presents the findings of an extremely high BAC death along with other depressants. The results obtained during the analysis were much higher than what is expected during a typical post-mortem analysis, leading to multiple different sample types being used to confirm the excessively high BAC and other results. In cases such as this one, where the decedent has significant medical issues as well as significant toxicological findings, the toxicological results can greatly affect the determination of the cause and manner of death.

Methods: All matrices were analyzed for ethanol and other volatiles by Headspace Gas Chromatography with Flame Ionization Detector and Headspace Gas Chromatography with Flame Ionization Detector and Mass Spectrometer. Presumptive screening for cannabinoids and barbiturates by immunoassay and a qualitative analysis for approximately 320 drugs by LCQTOF was performed on the central blood. Quantitative analysis on all matrices for drugs other than alcohol were performed using a protein precipitation followed by a DPX® tip clean-up prior to analysis by a Waters Acquity-UPLC and XeVo TQ-S dual mass spectrometer.

Results: The autopsy findings included: pill/capsule residue in the stomach, mild pulmonary congestion, minimal edema, severe coronary atherosclerosis, pleural adhesions on the right lung, acute visceral congestion, a surgically absent appendix and corresponding scar, and multiple scars on the upper left extremity.

Specimen	Doxylamine	Zolpidem	Diphen-hydramine	Acetamin-ophen	Methorphan	Ethanol
Central Cavity Blood	558 ng/mL	592 ng/mL	2670 ng/g	>32000 ng/mL	270 ng/mL	0.862% w/v
Brain Homogenate	536 ng/g	375 ng/g	5966 ng/g	70335 ng/g	784 ng/g	2.383% w/v
Liver Homogenate	2062 ng/g	996 ng/g	19822 ng/g	141311 ng/g	2892 ng/g	0.740% w/v
Peripheral Blood	392 ng/mL	540 ng/mL	2079 ng/mL		229 mg/mL	2.138% w/v
Stomach Contents	< 22 mg	< 9 mg	< 22 mg	> 6700 mg	< 2.2 mg	7.608% w/v
Urine						0.086% w/v
Vitreous Humor						0.753% w/v

Other findings include bromazepam, caffeine, chlorpheniramine, dextrorphan/levorphanol, metoprolol, N-desmethylmirtazapine, and zolpidem phenyl-4-carboxylic acid.

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Discussion: The tested post-mortem ethanol result of 0.862 % w/v is more than double the generally accepted LD50 for ethanol of 0.40 % w/v. As a result of the extremely high ethanol levels, the over-the-counter depressants - doxylamine and diphenhydramine- and the prescription depressant zolpidem, the deceased would likely have been experiencing extreme depressant effects prior to death, although it's unknown whether any or all of the ethanol and drugs were consumed prior to driving to the side of the freeway. Without all of these toxicological results, the cause of death may have been attributed to atherosclerosis. However, the cause of death was determined to be acute polydrug intoxication, the combined toxic effects of ethanol, diphenhydramine, doxylamine, zolpidem, methorphan, dextrorphan/levorphanol, acetaminophen, bromazepam, chlorpheniramine, metoprolol and N-desmethylmirtazapine, and severe coronary atherosclerosis. The manner of death was determined to be suicide.

P041

Toxicological profile of diquat and bromide ion concentrations in blood in a fatal poisoning case

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Abstract

Introduction: Herbicide poisoning commonly involves both paraquat and diquat (DQ); DQ poisoning alone is less frequently reported, and especially rare in Japan. Here we present a case of an 82-year-old female who was admitted to the emergency department (ED) after attempted suicide by ingesting 150 mL of herbicide (33% diquat dibromide solution). Following gastric lavage and activated charcoal administration, she was admitted to the intensive care unit (ICU) where hemodialysis (HD) was performed twice over 12 hours. Despite intensive care, she died from multiple organ failure approximately 40 hours after transport. We investigated the relationship between the blood concentrations of DQ, DQ metabolites, and bromide ion (Br-) relative to hemodialysis treatment course in a fatal DQ poisoning case.

Objectives: To investigate the time profiles and relationship between DQ, DQ metabolites, and Br- concentrations in blood in a fatal diquat poisoning case.

Methods: Diquat dibromide monohydrate was purchased from GL Sciences (Tokyo, Japan). Diquat monopyridone (DQ-M) and diquat dipyridone (DQ-D) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Sodium chloride, sodium tetraborate decahydrate, and potassium thiocyanate were from FUJIFILM Wako Pure Chemical Co. (Osaka, Japan). Potassium bromide was from Spectra-Tech, Inc. (Stamford, CT, USA). Serum samples were prepared by acetonitrile (ACN) protein precipitation. DQ and DQ-metabolites were analyzed on a 4000QTRAP (AB Sciex, Framingham, MA, USA) mass spectrometer coupled to a Shimadzu Prominence UFLC LC System (Shimadzu Co., Kyoto, Japan) using an Atlantis HILIC Silica (50 mm x 2.1 mm ID x 3 µm) column (Waters, Milford, MA, USA) under gradient elution using (A) water, 50 mM NH_4HCO_2, 0.5% formic acid and (B) 25:75 water:ACN, 50 mM NH_4HCO_2, 0.5% formic acid mobile phases and positive ESI detection in the MRM mode. Bromide was analyzed on an Agilent 7100 Capillary Electrophoresis System using untreated fused-silica capillaries (64.5 cm total length, 56 cm effective length, 50 µm ID) (Agilent Technologies, Santa Clara, CA, USA), in reverse polarity mode under a constant voltage of 20 kV with direct UV detection at 200 nm.

Results: Quantitative results are shown in Table 1. Blood DQ levels were in agreement with the qualitative colorimetric test performed prior to quantitative analysis. The two DQ metabolite concentrations were inversely correlated to DQ concentration, while Br- concentration was proportionally correlated to DQ over the time-course profile.

Table 1. Quantitated concentrations of DQ, DQ-M, DQ-D, and Br- in blood.

Time after ingestion (hrs)	Note	Concentration in blood (µg/mL)			
		DQ	DQ-M	DQ-D	Br-
2.5	Pre-HD#1	75	0.01	0.02	493
6.5	Post-HD#1	8.4	0.11	0.08	27
13		12	0.04	0.09	49
14	Pre-HD#2	12	0.05	0.17	47
18	Post-HD#2	1.5	0.05	0.06	<LOQ
37.5	Prior to death	2.8	0.02	0.14	<LOQ

Discussion: While HD treatment seemed to have reduced the DQ concentration significantly, re-elevation of the blood DQ level suggests that it was a temporary relief not enough to prevent the patient from going into multiple organ failure. The ingested herbicide contained 33% diquat dibromide thus bromide ion would have also been absorbed into the body

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along with DQ. Bromide half-life in human body is about 12 days, much longer than DQ which is rapidly eliminated within 48 hours of ingestion. Bromide-to-diquat ratio in blood was greater than 3 in all blood samples depicting that Br- remains in the system longer than DQ, which could pose a risk to prolongued Br- exposure leading to further intoxication if the patient had survived. This is the first report investigating the time-course profiles and relationship of blood DQ, its primary metabolites, and bromide ion in a DQ-containing herbicide poisoning case.

P042

Analysis of Drug-infused Papers by ASAP-MS

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Abstract

Introduction: Drug misuse within UK secure institutions, e.g., prisons, is prevalent and a major concern as it contributes to increased levels of aggression and violence. It has been reported that letters, and other materials, impregnated with drug substances have been sent to individuals. While traditional drug substances continue to be widespread, the ongoing emergence of potent novel psychoactive substances (NPS) has significantly exacerbated the issue. Reducing drug access is a key consideration in the overall strategy to reduce drug misuse; testing materials received by individuals, may assist in this process.

Objectives: To assess the potential of RADIAN™ ASAP Mass Detector, a compact device based on Atmospheric Solids Analysis Probe-Mass Spectrometry (ASAP-MS), as a simple, rapid, screening tool for the detection and semi-quantitation of drug substances in suspect papers. ASAP-MS analysis is a direct analysis technique yielding mass spectrometry data without chromatographic separation.

Methods: Twenty suspect paper samples (1cm^2 squares) were extracted by sonication in 500 µL methanol and sampled by dipping a glass capillary into the supernatant for 10 seconds, before loading into the ASAP device. Mass detection was performed using full scan MS (*m/z* 50-650), at a temperature of 600°C. To further enhance specificity, data was acquired simultaneously at four differing cone voltages (15, 25, 30, 50V), which led to the generation of characteristic product ions. Data was processed by LiveID™ software which matched the acquired data to a spectral library (Waters) and calculated average match scores for each detected compound. A match score of 850 (from max.1000) was used as the minimum reporting criteria for a positive detection. Extracts were also analysed using an established QTof-based screening method following a further dilution (1:2000).

Results: ASAP-MS analysis of the paper samples led to a positive detection of MDMB-CHMICA (n=5), 5F-AKB48 (n=10) or both (n=3) in 18 of the paper samples. Match scores were based on the mean of the four cone voltages and ranged from 881 to 989. Two samples did not return any positive matches and were deemed negative. Overall there was good agreement with QTof analysis; some additional positive detections by the latter technique were likely due to large differences in analytical sensitivity between instruments. Work was extended to assess the feasibility of performing a semi-quantitative analysis of drugs in papers using ASAP-MS, facilitated by incorporation of an internal standard (Molsidomine) into a second extraction, with a calibration series prepared with 1 mg/mL certified reference materials, ranging from 0.25-50 µg/cm^2. Paper samples with positive detections for MDMB-CHMICA were found to contain concentrations ranging from approximately 47-3000 µg/cm^2 (n=5), employing a dilution protocol. Paper samples with positive detections for 5F-AKB48 were found to contain concentrations ranging from approximately 448-2150µg/cm^2 (n=5), again, employing a dilution protocol.

Conclusions/Discussion: ASAP-MS is a rapid (<2min) and simple screen for drugs in paper samples and may be an effective tool to reduce access to drugs in secure institutions.

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POSTER

P043

Analysis of carboxy-THC in hair using UPLC-MS/MS

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Abstract

Introduction: Delta-9-tetrahydrocannabinol (THC) is the main psychoactive component of cannabis and produces several metabolites including 11-nor-9-carboxy-Δ9- tetrahydrocannabinol (carboxy-THC). After a positive identification of THC in hair, carboxy-THC can subsequently be analyzed to comply with the Society of Hair Testing (SoHT) requirements. However, this analysis is very challenging, as carboxy-THC is found at low pg/mg concentrations, with typically limited sample availability, thus high sensitivity analytical techniques are required.

Objectives: The aim was to consider a robust method for the analysis of carboxy-THC in hair, that meets the routine guidelines for the confirmation cut-off concentrations recommended by the SoHT.

Methods: Decontaminated scissor minced hair samples (including blond, brown, black and coloured hair) were weighed (25 mg) into centrifuge tubes, the internal standard (carboxy-THC-d3) was added along with M3 Reagent (from Comedical s.r.l., Trento, Italy). For calibration standards cTHC (0.2-10 pg/mg) was also added. Samples were incubated for 60 min at 100 °C, cooled, and loaded onto OasisTM PRiME HLB 30 mg Cartridges. Samples were washed with acetonitrile solution then hexane. carboxy-THC was eluted with acetonitrile/methanol and following evaporation samples were reconstituted in a methanol solution and transferred to vials for analysis.

Carboxy-THC was separated using a UPLC™ system gradient of ammonium fluoride and methanol. Two MRM transitions for carboxy-THC were monitored using the Xevo™ TQ-Absolute Mass Spectrometer i.e., m/z 343.1 >191.0, and m/z 343.1 > 245.1, the internal standard (carboxy-THC-d3) was also monitored using the transition m/z 346.1 > 248.1.

Results: The linearity of the assay was investigated over the range 0.2 to 10 pg/mg, with R^2 >0.99 achieved. carboxyTHC at 0.2 pg/mg (confirmation cut-off recommended by SoHT) is reported, with signal to noise >15 achieved for the chromatograms for both the quantifier and qualifier MRM transitions.

The robustness of the assay was investigated considering the analysis of mixed hair samples (n=5), each sample extract was injected in triplicate (total %RSD (% relative standard deviation) = 8.4 and average within sample %RSD = 1.8).

No detectable carryover was observed, assessed after the injection of a high-level spiked hair standard at 5 pg/mg followed by a blank (injection solvent) injection.

Discussion: After optimization of the chromatographic, mass spectrometer, and sample preparation conditions, using the ACQUITY™ UPLC I-Class system with Xevo TQ Absolute Mass Spectrometer has demonstrated, the ability to obtain the required analytical sensitivity to detect carboxy-THC in hair at sub pg/mg concentrations.

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POSTER

P044

Comparison of Data-Independent and Data-Dependent Techniques for Forensic Toxicology Screening Analysis

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Abstract

Introduction: High resolution mass spectrometry has gained popularity for broad toxicological screening. In addition to monitoring the precursor accurate mass, common analytical practice is inclusion of retention time (RT) and additional mass spectrometry data generated through data-independent (DIA) or data-dependent (DDA) techniques.

Objective: The aim of this study was to compare these methods with respect to data generated e.g., richness of information and screening efficiency e.g., detection accuracy and analytical sensitivity.

Methods: Urine samples were prepared by dilution. Analysis was performed using an ACQUITY™ UPLC™ I Class system combined with a Xevo™ G3 QTof system (Waters). The same 15-min chromatographic method was applied for all techniques. For DIA, MS^E acquisition was applied; this mode facilitated collection of full MS spectra at two collision-cell voltages (6eV and a ramped 10-40eV). Two approaches were evaluated for DDA: the first (DDA-1) used a minimum threshold response during a MS survey scan; exceeding this, triggered MS/MS, the second (DDA-2), used the same threshold but also included a list of 25 precursor masses to preferentially target key analytes for MS/MS analysis. Data from all techniques were compared with an established toxicology library based on RT and accurate mass fragment data for >2000 analytes.

Results: The low voltage of MS^E (DIA) showed abundant precursor masses, while the ramped-voltage facilitated fragmentation of the precursor(s). Visually, spectra for co-eluting analytes contained fragment ions from each component. MS/MS spectra generated by DDA were less complex, containing only the fragment ions arising from the quadrupole-selected precursor. For DDA, several trigger thresholds were evaluated; a threshold of 100,000 counts gave the best results. Analysis of 20 pre-characterized authentic urine samples by MS^E, DDA-1 and DDA-2, resulted in the correct identification of 94%, 86% and 82% of expected analytes, respectively.

Discussion and Conclusions: The DIA method of MS^E was easier to implement as optimization of trigger thresholds was unnecessary. Despite the more complex spectra, this mode resulted in improved detection of expected drugs. Despite the more complex spectra, MS^E urine sample data was processed 4 times quicker than the equivalent DDA data, therefore high throughput laboratories would be able to process complete unrestricted MS^E data faster than a DDA data set. The few false negatives by MS^E were associated with analytes at very low concentration. False negatives by both DDA methods appeared to be due to triggering conflicts, particularly where co-elution was evident.

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POSTER

P045

Urinary Forensic Toxicology Data Independent Analysis Screening: Using High Resolving Power Multi-Reflecting Time-of-Flight Mass Spectrometry

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Abstract

Introduction: Forensic laboratories are frequently required to perform broad screening techniques on complex biological samples to identify drugs of abuse, prescribed agents and other toxicants. Broadband data-independent analysis (DIA) has been previously applied for non-targeted screening of forensic samples. The technology was applied using high resolution mass spectrometry (HRMS at 20,000 FWHM) and enabled collection of an unbiased dataset, providing a complete profile of the sample complement, including precursor and fragment ions.

Objective: To demonstrate enhancement of DIA specificity, through use of a high mass resolving power (>200,000 FWHM) hybrid quadrupole multi-reflecting time-of-flight (Q-MRT) MS. Through the analysis of anonymised urine samples [JC1] to highlight enhanced analyte ion selectivity in complex matrices, improved mass accuracy and analyte identification confidence.

Methods: Broadband DIA using alternate scanning precursor/fragment ion data acquisition was performed using Q-MRT MS (system resolving power >200,000 FWHM) acquired using positive electrospray mode of ionization. An acquisition rate of 10Hz and a collision energy ramp of 10-40eV were applied. Human urine samples (n=10) were analysed, using reversed phase chromatography, comprising gradient elution of a 150mm x 2.1mm, 1.8µm C18 column. The column was maintained at 50ºC and eluted with a mixture of (A) 5 mM ammonium formate in water, pH 3.0 and (B) acetonitrile delivered at 0.4mL/min. The run time was 15 minutes and an injection volume of 5µL was utilised. Authentic urine samples were diluted 1:10 (into water) prior to the analysis.

Results and Discussion: Non-targeted data acquisition has been performed for a forensic system suitability test mix (SST) and a series of authentic human urine samples. Post-acquisition processing involved comparison with a comprehensive library of over 1900 toxicologically relevant compounds (Waters), including illicit, prescription and OTC drugs, and pesticides.

For the 10-component SST mix (250pg/µL) mass error (RMS) of 522ppb was obtained, where data processing tolerances of tr (0.35min) and mass accuracy of +/2ppm (previously +/-5ppm), diagnostic fragment ion count ≥1 and expected fragment ion tolerance of 0.2mDa (previously 2mDa) were applied. All SST analytes were identified using the more stringent data processing parameters. As an example, for clozapine, precursor m/z 327 [397ppb (184,000 FWHM)] and fragment ions m/z 270 [411ppb (190,000 FWHM)], m/z 227 [236ppb (183,000 FWHM)], m/z 192 [382ppb (178,000 FWHM)], m/z 227 [236ppb (183,000 FWHM)], m/z 84 [471ppb (130,000 FWHM)] have been observed.

The same parameters were also applied when processing the urine samples and comparing against the library. The high sub-ppm mass measurement specificity attained for the SST constituents afforded increased confidence for identifications made in the authentic samples, which included illicit and prescribed drugs, their metabolites, as well as dietary/endogenous constituents. For example, in authentic sample “103”, methadone [m/z 310 (290 ppb)] EDDP [m/z 278 (350 ppb)], methamphetamine (m/z 150 (-520 ppb)), morphine (m/z 286 (930 ppb)), normorphine [272 (m/z (920 ppb)], codeine [m/z 300 (-290 ppb)], norcodeine [m/z 286 (-570 ppb)], nicotine [m/z 163 (-460 ppb)], cotinine [m/z 177 (-1090 ppb)], caffeine [m/z 195 (110 ppb)], and theophylline [m/z 181 (960 ppb)], were identified.

POSTER

P046

Evaluating the stability of promethazine in solution with the addition of hydrochloric acid

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Abstract

Introduction: Promethazine is an antihistamine often prescribed for allergies and motion sickness. It is a phenothiazine derivative, meaning its structure is light sensitive and requires storage in amber glassware when used as a reference material. Current laboratory practice is to store promethazine in-house working calibrator and control solutions in methanol in amber containers. The stability of in-house reference solutions is often short–lived, and extending the life of these solutions will benefit the toxicology laboratory.

Objectives: The goal of this project was to determine if adding hydrochloric acid during the preparation of in-house calibrators stabilizes promethazine in solution when compared to standard non-acidic in-house calibrator solutions.

Methods: Promethazine working calibrator solutions were prepared in methanol and 1% 1N hydrochloric acid in methanol each month, and then were used to prepare promethazine calibrators ranging from 100 ng/mL to 4000 ng/mL. The liquid-liquid extraction at each time point contained the freshly prepared calibrator solutions along with any prepared calibrator solutions from the previous month(s). The extracts were injected within 24 hours on an Agilent 6400 series LCMSMS using a C18 column and mobile phase consisting of 7mM ammonium formate + 0.05% formic acid in water (A) and acetonitrile (B). Comparison of standard and acidic calibrators were evaluated at 1, 2, 3, and 4 months after the initial solution preparation using a paired t-test.

Results: The comparison of the response ratios at 1, 2, and 4 months after the initial lot preparation shows no statistical difference between the standard calibrators and the acidic calibrators, while the response ratios at 3 months show a statistical difference between the standard and acidic solutions.

Discussion: Adding hydrochloric acid to methanol used in the preparation of promethazine calibrators did not have an effect on the stability of the solutions 1 month and 2 months after preparation when stored in amber jars. Three months from initial preparation, there is a statistical difference indicating the acid has the potential to improve the stability of calibrator solutions over a longer period of time; however, when evaluating the calibrator solutions at 4 months, there was no difference between the acidic and standard solutions. It would be expected that if the acid improved stability then the 4 month statistical test would also indicate a difference. However, fewer data points were available at the 4 month time point, possibly impacting the outcome. Additional months of data collection is needed to determine the effect of acid on promethazine stability.

POSTER

P047

Pediatric Deaths: A 10-year review of cases handled by Onondaga County Medical Examiner's Forensic Toxicology Laboratory.

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Abstract

Introduction: A regional office, the Onondaga County Medical Examiner's Office (OCMEO) serves the surrounding counties, particularly Cayuga, Madison, Oneida, and Oswego counties, with medical examiner services. The Toxicology Laboratory, a part of the OCMEO, identifies and quantitates drugs, alcohol and poisons and plays a pivotal role in the determination of the causes surrounding pediatric deaths and shedding light on the complexities of pediatric mortality. This study presents a 10-year review of pediatric deaths handled by OCMEO, examining the role of toxic substances in fatal outcomes.

Objective: Through an in-depth examination of postmortem toxicology, medical examiner and investigator reports, this study seeks to better understand the unique challenges faced in forensic investigations involving children. Understanding the circumstances and causes of these deaths is critical for improving public health programs and mitigating similar situations.

Methods: A database search of OCMEO pediatric (0-18 years old) cases was undertaken from January 1, 2013 to May 8, 2023. Comprehensive toxicology screening is performed for all pediatric cases and quantitation is carried out as necessary.

Results: The OCMEO examined 527 pediatric deaths in total between 2013 to May 8, 2023, or 4.8% of the total OCMEO cases. Unsafe sleep environments, motor vehicle accidents, and gunshot wounds accounted for 19.3%, 15.1% and 8.7% of all pediatric deaths, respectively. Drug, alcohol, or poison deaths ranked ninth among pediatric causes of mortality, accounting for 4.7% of all pediatric deaths. An opioid was identified in 19 of the 25 drug, alcohol, or poison deaths. The manner of death for these cases was accident, suicide, and homicide. The two homicide cases featured pediatric decedents aged two years old and younger.

Discussion: This 10-year review of pediatric deaths handled by the OCMEO underlines the impact of toxic substances in fatal outcomes and provides valuable information for enhancing public health policies and preventing similar tragedies. The findings emphasize the significance of addressing key factors to pediatric death, which are unsafe sleep environments, motor vehicle accidents, and firearm-related incidents as major contributors to pediatric mortality. Furthermore, the inclusion of opioids in drug and toxin deaths, as well as homicides among pediatric deaths, highlight the critical need for focused interventions and comprehensive initiatives to safeguard children and prevent these avoidable deaths.

POSTER

P048

Evaluating Methcathinone and Pseudoephedrine Levels in Over-the-Counter Drugs and Corresponding Urine Samples

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Abstract

Objective: New psychoactive substances (NPS) are evolving rapidly in the recreational drug market. Synthetic cathinone (SC), a type of NPS, is cheaper and possesses stimulant and hallucinogenic properties. Therefore, it is considered an alternative to 3,4-methylenedioxymeth/amphetamine (MDMA) or amphetamine. Methcathinone (MC) is a SC with a chemical structure and pharmacological properties similar to amphetamine. In the late 1920s, German and French chemists were the first to synthesize MC as an intermediate compound during the production of ephedrine

Monitoring the abuse of illicit drugs, including MC, is of great importance to drug control authorities. Urine drug tests are a useful method for monitoring the illicit drug market.

The chemical structure of MC is similar to pseudoephedrine/ephedrine. Pseudoephedrine and ephedrine are diastereomers. MC differs from pseudoephedrine/ephedrine only by the β-ketone substituent. MC can be easily produced by the oxidation of pseudoephedrine/ephedrine, for example, with acid and potassium permanganate as the oxidant. Pseudoephedrine or ephedrine is a common component of over-the-counter drugs for the common cold or allergic rhinitis in Taiwan. We found some cases where MC was detected in urine samples, but they used over-the-counter drugs without any evidence of MC use. Although SC use is popular in the worldwide , the lack of MC seizures suggests that the MC detected in these cases could not be accounted for by the use of MC.

The objective of this study was to check the concentrations of MC and pseudoephedrine/ephedrine in over-the-counter drugs containing pseudoephedrine/ephedrine. We also investigated whether the consumption of over-the-counter drugs could result in relevant urine concentrations of MC and pseudoephedrine.

Methods: The concentrations of pseudoephedrine/ephedrine and MC were analyzed by LC-MS/MS in four different brands of over-the-counter drugs containing 60 mg of pseudoephedrine per pill. Twelve healthy volunteers participated in the experiment. Each subject consumed the over-the-counter drugs containing 60 mg of pseudoephedrine once. After ingesting the capsule, they were allowed to drink a maximum of 100 ml of water within the next hour. Spot urine samples were collected at time points 0,2 6,12 and 24 hours after ingestion.Our experiment is designed to simulate daily life, so there are no restrictions on the subjects' liquid/food ingestion during the experiment period.

Results: Measurement of the over-the-counter drugs revealed a concentration of 45.95-52.55 mg of pseudoephedrine/ephedrine (76.58-87.58% of the declared content) and 0.14-2.95 μg of methcathinone (Table). The peak levels of MC were detected six hours after the ingestion of the different brands of over-the-counter drugs in all cases. No MC was detected 24 hours later.The peak concentration of MC in urine exceeded the cut-off (1 ng/ml) in all cases (1.01-6.38 ng/ml).

Brand	Methcathinone(mg)	Pseduoephedrine(mg)	Methcathinone/pesduoephedrine
Brand1	$4.1x10^{-4}$	52.55	$7.7x10^{-6}$
Brand2	$2.95x10^{-3}$	45.95	$6.5x10^{-5}$
Brand3	$1.83x10^{-3}$	46.57	$3.9x10^{-5}$
Brand4	$1.79x10^{-3}$	53.78	$3.3x10^{-5}$
In each brand, the content of pseudoephedrine is labeled as 60 mg per capsule			

POSTER

Conclusions: Ingesting a single dose of over-the-counter drugs containing 60 mg of pseudoephedrine may result in relevant amounts of MC in urine samples. Two urine samples revealed MC values above the threshold (10 ng/ml) 6 hours after ingestion. These low MC concentrations may be attributed to trace amounts of MC present in the over-the-counter drugs containing MC (0.14-2.95 µg). There is a reasonable risk for consumers of these over-the-counter drugs to test positive for MC.

P049

The Benefits of 2.1 mm Internal Diameter Analytical Columns for the Analysis of Drugs of Abuse by LC-MS/MS

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Abstract

Introduction: The biphenyl phase offers advantageous selectivity compared to a C18 column for drugs of abuse panels, but choosing the right column dimension is paramount to obtain robust and accurate data. Every column dimension can be advantageous in different scenarios, but clinical labs are generally all working towards the same goals: high throughput, low sample volume, good sensitivity, and low cost. In this work, the advantage of 2.1 mm internal diameter (ID) columns is discussed and demonstrated for the analysis of drugs of abuse.

Objectives: The primary objective is to demonstrate the advantage of the 2.1 mm ID column dimension for the analysis of drugs of abuse by LC-MS/MS.

Methods: Two methods were developed to analyze common isobars in drugs of abuse panels in urine on Raptor Biphenyl columns using methanol and water modified with 0.1% formic acid and column oven set to 45 ^{o}C. One method used a 50 x 2.1 mm column with a flow rate of 0.6 mL/min and the other used a 50 x 4.6 mm column with a flow rate of 0.9 mL/min. Both methods used gradient conditions with a total cycle time of 9 minutes. A lifetime study was conducted by performing 1,000 total injections of samples prepared through a common dilute-and-shoot approach.

Results: The two methods were compared for efficiency, sensitivity, resolution (Rs), consumption of mobile phase, and robustness. Buprenorphine was used to demonstrate sensitivity differences between the two different column dimensions. It was found that the 2.1 mm ID column produced twice the signal response of the 4.6 mm ID column when injection volume is held constant. Twice the amount of sample had to be injected on the larger-bore column to produce the same signal response, thus introducing the chromatography to greater matrix effects with more impact to instrument cleanliness. A number of analytes, including 9 pairs of isobars commonly found in the analysis of drugs of abuse were evaluated. The resolution of each pair of isobars demonstrated in these experiments was calculated for the 2.1 mm ID column and each pair was able to achieve Rs ≥ 1.5. A lifetime study of the 50 x 2.1 mm ID column in matrix was able to illustrate the robustness of the column dimension over 1,000 injections.

Conclusion/Discussion: In summary, the 2.1 mm ID column was able to demonstrate superior sensitivity while consuming less resources and minimizing impact to the MS while still providing adequate resolution of isobars and excellent column robustness.

POSTER

P050

Determining the optimum derivatizing agents and parameters to improve the detection of anabolic-androgenic steroids

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Abstract

Introduction: Anabolic-androgenic steroids (AASs) typically require derivatization to increase the volatility and/or thermal stability of compounds to make the compound more amenable for gas chromatography-mass spectrometry (GC-MS) analysis. The typical method of derivatization used for AASs is trimethylation using MSTFA with the occasional addition of various catalysts, although some methods also utilize BSTFA with 1% TMCS. Typically, derivatization of AASs via the addition of MSTFA is usually carried out by heating at 60°C for 1hr. Publications have used microwave assisted derivatization (MAD) for steroids which has shown to significantly decrease long derivatization times.

Objective: To determine the yields of AASs subjected to traditional derivatization methods and microwave-assisted derivatization (MAD).

Methods: To 4 mL vials (n=5), 100 µL (10 ug/mL) of methanolic solutions of 17α-methyltestosterone, 19-norandrosterone, 2α-methyl androsterone, 6β-hydroxy metandienone, boldenone, clenbuterol, clostebol, DHEA, drostanolone, epitestosterone, fluoxymesterone, testosterone, mestanolone, mesterolone, metandienone, methylclostebol, oxandrolone, oxymetholone and diazepam (internal standard) were added. Diazepam was chosen as the internal standard as this does not undergo derivatization. Vials were then evaporated to dryness using a Genevac (DNA-23050-A00). Samples (n=5) were then reconstituted in 50 µL MSTFA, BSTFA +1% TMCS or MSTFA/NH4I/ethanethiol before being microwaved at 700W using an iGENIX microwave (Model: IG2071) for 30s, 1mins, 3mins or 5mins. Additionally, the AAS mixes were subjected to room temperature (control), and conventional heating at 37°C, 50°C, 75°C, 90°C utilizing a TECHNE Dri-Block DB-2D for 15mins, 30mins,1hr and 2hrs. After heating, the AAS mixes were analyzed using an Agilent 7820A GC (column: Zebron ZB-1 (30m x 0.25mm, 0.25µm)) coupled with a mass spectrometer 5977B MSD (Agilent Technologies Inc., UK). The average peak area ratio (APAR) was calculated using the peak area response for diazepam and each analyte, allowing for a degree of normalization between parameters, as well as accounting for any inter- and intraday differences. SPSS (version 28) was used to conduct a MANOVA to determine any statistical significance of results.

Results: MSTFA/NH4I/ethanethiol outperformed MSTFA and BSTFA +1% TMCS ($p<0.05$) in almost all circumstances for all analytes. The overall optimal method was found to be MSTFA/NH4I/ethanethiol incubated for 15mins at 37°C using the traditional heat block. It was found that longer incubation times resulted in lower average peak area ratios (APARs) using the traditional heating block and microwave. As microwave samples were subjected to longer heat times the coefficient of variation also increased. This was potentially due to the difference in temperature experienced by the samples due to varied positioning within the microwave, meaning each vial was exposed to different paths of microwave deflection.

Discussion: The results of this project show that AASs can be successfully derivatized using MAD, producing consistently detectable APAR comparable to traditional heating block incubation methods, in a much shorter time frame. Overall, MSTFA/NH4I/ethanethiol was shown to produce the highest APARs for the AASs derivatized. The use of MAD significantly reduces sample preparation time resulting in faster GC-MS methods for the routine detection and analysis of these analytes.

Brand	Methcathinone(mg)	Pseduoephedrine(mg)	Methcathinone/pesduoephedrine
Brand1	4.1×10^{-4}	52.55	7.7×10^{-6}
Brand2	2.95×10^{-3}	45.95	6.5×10^{-5}
Brand3	1.83×10^{-3}	46.57	3.9×10^{-5}
Brand4	1.79×10^{-3}	53.78	3.3×10^{-5}
In each brand, the content of pseudoephedrine is labeled as 60 mg per capsule			

POSTER

P051

Investigations into the Human Metabolism of the Minor Cannabinoid Cannabichromene

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Abstract

Introduction: Cannabichromene (CBC) is a phytocannabinoid found in Cannabis. There are over 111 identified phytocannabinoids, and CBC is among the most prevalent cannabinoids behind Δ^9-THC, CBD, CBN, and CBG. As cannabis use becomes more commonplace both medicinally and recreationally, it is crucial to understand the metabolism of cannabinoids, their activity, and potential risks.

Objectives: Previous studies have identified oxidized CBC metabolites in a variety of animal models and dihydroxylated metabolites in rabbits, though no studies to date have identified metabolites in humans. The present study aims to prepare, isolate, and characterize metabolites of CBC utilizing human liver microsomes (HLMs) and recombinant cytochrome P450s (CYP450).

Methods: CBC was incubated with HLMs or CYP450s for 40 minutes, then the reaction was quenched with ice cold acetonitrile. The slurry was centrifuged, and the supernatant was analyzed. Metabolite characterization and structure elucidation was performed utilizing high-resolution mass spectrometry on a Quadrupole Time-of-Flight (Q-TOF), MSn on ion trap, and gas chromatography mass spectrometry (GCMS) to generate and compare fragmentation patterns between CBC and the metabolites. Nuclear magnetic resonance (NMR) spectroscopy was used to determine the structure of the major metabolite of CBC. A panel of 11 recombinant CYP450s (CYP1A1, CYP1A2, CYP3A4, CYP3A5, CYP3A7, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, CYP2E1) was used to identify the main metabolizing enzymes and subsequent products.

Results: Oxidation in one position of CBC represented nearly 70% of metabolites generated by human liver microsomes, while oxidation in two positions of CBC represented nearly 20% of metabolites generated. CYP3A and CYP1A were found to be the major CBC metabolizing subfamilies of CYP450s in the human liver.

Discussion: Future work will focus on the structural elucidation of additional metabolites paired with assigning specific metabolites to CYP450s which will provide insight into potential drug-drug interactions. Furthermore, major metabolites will be tested for potential biological activity and toxicity.

POSTER

P052

Evaluation of a Quantitative Analysis Method for Tetrahydrocannabinol Isomers in Biological Matrices

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Abstract

Introduction: As of late, forensic toxicology laboratories have been grappling with the emergence of tetrahydrocannabinol isomers. Traditional methods for the identification and quantitation of cannabinoids typically includes the evaluation of Δ^9-tetrahydrocannabinol (Δ^9-THC) and its metabolites in biological matrices. Upon analysis of additional tetrahydrocannabinol isomers, laboratories often find co-elution or minimal separation between Δ^9-THC, exo-THC, Δ^8-THC, Δ^{10}-THC, and $\Delta^{6a,10a}$-THC. These emerging isomers are commonly observed in the seized drug community in manufactured cannabis products (e.g., edibles). Trends within seized drugs and legislative changes incorporating tetrahydrocannabinol isomers dictates the need for change within forensic toxicology. Traditional methods require adaptation to the ever-changing climate surrounding tetrahydrocannabinol. Additional method development with subsequent validation to meet *ANSI/ASB Standard 036, Standard Practices for Method Validation in Forensic Toxicology* is often required.

Objectives: This project aims to expand the scope for cannabinoid testing in forensic toxicology laboratories to include phytocannabinoid constituents and metabolites and validate the newly developed extraction and analytical parameters to meet *ANSI/ASB Standard 036, Standard Practices for Method Validation in Forensic Toxicology.*

Methods: A dual chromatographic column method was developed and optimized for the separation of tetrahydrocannabinol isomers. An Agilent Technologies 1290 Infinity liquid chromatograph was coupled independently to both a 6460 and 6470 quadrupole mass spectrometer for development and validation. Two independent chromatographic methods were developed using different analytical columns, mobile phase conditions, chromatographic gradients, and flow rates. The qualitative analytical method utilized an Agilent Technologies Poroshell 120 PFP 3.0 x 100 mm, 2.7 µm column held at 50°C. The quantitative analytical method used an Agilent Technologies Poroshell 120 EC-C18 3.0 x 50 mm, 2.7 µm column held at 50°C. The dual column methodology was used to enhance the separation of tetrahydrocannabinol isomers.

The sample preparation procedure consisted of supported liquid extraction (SLE) using 0.5 mL of biological specimen. The sample was acidified with 200 µL of formic acid in water prior to placement on the SLE cartridge. Specimens were incubated for 5 minutes prior to the addition of ethyl acetate (3.0 mL). After collection, n-hexane (3.0 mL) was added to each cartridge. Samples were evaporated to dryness at approximately 50°C prior to reconstitution in 50 µL of methanol.

Results: The optimized method was validated for quantitation of Δ^9-THC, (±)-11-hydroxy-Δ^9-THC (Δ^9-OH-THC), (±)-11-nor-9-carboxy-Δ^9-THC (Δ^9-carboxy-THC), (-)-Δ^8-tetrahydrocannabinol (Δ^8-THC), and cannabidiol to meet *ANSI/ASB Standard 036, Standard Practices for Method Validation in Forensic Toxicology*. All other isomers were validated for qualitative identification. During validation, samples were evaluated on both analytical methods to ensure congruence in results. The calibration range was 1/2/5 ng/mL to 100/200/500 ng/mL (Δ^9-THC, Δ^8-THC/Δ^9-OH-THC, cannabidiol/Δ^9-carboxy-THC). All compounds were within ±20% for bias and precision when evaluating pooled fortified samples of blank blood, antemortem blood, and postmortem blood. Significant ionization suppression (>25%) was noted for antemortem blood, postmortem blood, and urine requiring additional matrices to be added to the estimated limit of detection and lower limit of quantitation experiments.

Further, processed samples were evaluated for stability over a seven day period. Given the volatility of the reconstitution solvent, a ratioed stability was performed and an overall stability up to five days post extraction was noted. Further interferences were evaluated including other isomers and derivatives of Δ^9-THC. The estimated limit of detection and lower limit of quantitation were assessed for each matrix type within the validation.

Discussion: An extensive validation was performed on the optimized SLE extraction with subsequent analytical analysis using liquid chromatography tandem mass spectrometry. The validated method ensures chromatographic separation between tetrahydrocannabinols providing enhanced identification of these isobaric compounds.

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Validation of traditional and designer benzodiazepines in blood by gas chromatography-tandem mass spectrometry (GC-MS/MS)

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Abstract

Introduction: Over the last several years, a growing number of designer benzodiazepines (DBZD) have been introduced into the recreational drug market. By slightly modifying function groups of traditional benzodiazepines at various sites, DBZD retain similar yet not fully understood pharmacological effects while evading the legal sanctions. Although they rarely cause death by themselves, both traditional and designer benzodiazepines can have severe toxicity when concomitantly used with opioids and alcohol, leading to an increased risk of death. Several liquid chromatography-tandem mass spectrometry (LC-MS/MS) methods have been reported for analyzing DBZD in biological matrices. However, gas chromatography-mass spectrometry (GC-MS) based techniques, extensively used for traditional benzodiazepines but scarcely reported for quantitative analysis of DBZD, remain the method of choice for many laboratories. Currently, Maryland OCME uses gas chromatography paired with nitrogen–phosphorus detector (GC-NPD) and single quadrupole mass spectrometry (GC-MS) to confirm and quantitate only five benzodiazepines (diazepam, nordiazepam, midazolam, alprazolam, and 7-amino clonazepam). The sensitivity for identifying DBZD is also significantly limited. Therefore, a more sensitive method (using in-house GC-MS/MS and derivatization) dedicated to a larger panel of benzodiazepines becomes necessary.

Objectives: The objective of this study was to expand OCME's scope of analysis, improve the data quality, and share a robust GC-MS/MS method with laboratories that are at least partially relying on GC-MS assays.

Methods: During method development, Agilent MassHunter Optimizer was utilized to select multiple reaction monitoring (MRM) transitions and collision energies for nine traditional benzodiazepines, seven DBZD, and 13 deuterated internal standards (ITSD) (Table 1). For validation, accuracy, precision, linear ranges, the limit of detection and quantitation (LOD/LOQ), carryover, interference, dilution integrity, and stability studies were conducted following ANSI/ASB Standard 036. Sample preparation included addition of internal standard to 1 mL whole blood, followed by protein precipitation with 2mL 5% ZnSO4. Samples were centrifuged at 3200 RPM for 10 minutes, and the supernatant was transferred and mixed with 2mL 0.2M sodium acetate butter (pH4.5) before loading on Tecan® Cerex® Trace-B SPE cartridges. The sample eluent was evaporated to dryness at ≤40°C, reconstituted with 50 µL of acetonitrile and 50 µL of MTBSTFA (1% TMCS), and incubated at 70 °C for 30 min.

The analysis was performed with an Agilent Technologies GC-MS 8890/7000D (Santa Clara, CA, USA) instrument equipped with a 7693A autosampler. Gas chromatographic separations were carried out using an HP-5 MS (30m X 0.25mmX 0.25 µm) silica capillary column from Agilent Technologies.

Table 1. List of benzodiazepine drug panel and deuterated internal standards used.

Traditional Benzodiazepines/ITSD	Designer Benzodiazepines/ITSD
Diazepam/ Diazepam-d_5	Flubromazepam/ Phenazepam-d_4
Nordiazepam/ Nordiazepam-d_5	Phenazepam/ Phenazepam-d_4
Midazolam/ Midazolam-d_4	Flualprazolam/ Flubromazolam-d_4
Oxazepam/ Oxazepam-d_5	Flubromazolam/ Flubromazolam-d_4
Temazepam/ Temazepam-d_5	Adinazolam/ Flubromazolam-d_4
Lorazepam/ Lorazepam-d_4	Etizolam/ Etizolam-d_3
Clonazepam/ Clonazepam-d_4	Bromazolam/ Bromazolam-d_5
Alprazolam/ Alprazolam-d_5	
7-amino Clonazepam/7-amino Clonazepam-d_4	

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Results: Calibration models were determined to be linear and weighted (1/x) for all analytes. The quantitative range was 1-160 ng/mL for flubromazepam and phenazepam, 5-160 ng/mL for the remaining DBZD, 10-400 ng/mL for lorazepam and clonazepam, 10-600 ng/mL for alprazolam and 7-amino clonazepam, and 25-1500 ng/mL for the rest of traditional benzodiazepines. The bias and precision for all analytes at the low, medium, and high QC concentrations are less than 20%. No interference was observed from the matrix and other commonly encountered drugs. However, interference on the deuterated ITSD from the corresponding analyte was observed and more evident for lorazepam and clonazepam than for other analytes at ULOQ.

Discussion: Generally, higher sensitivity and more symmetric peak shapes were observed for the TBDMS derivatized analytes. Because of the unique isotopic pattern of Cl, benzodiazepines typically have strong responses on M+3 and M+4 ions, rendering more significant interference on the d-3 and d-4 ITSDs. Therefore, either M+5 or M+6 ions were selected instead as the parent ion (midazolam-d4, lorazepam-d4, flubromazolam-d4, and etizolam-d3), or an alternative MRM was necessary (clonazepam-d4, 7-amino clonazepam-d4, and phenazepam-d4). Simultaneously increasing the spiked ITSD concentrations helped reduce the interference.

P054

Detection of the Substituted Cathinone Alpha-PiHP in Postmortem Toxicology Cases

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Abstract

Substituted cathinones, synthetic derivatives of the naturally occurring cathinone, have been one class of novel psychoactive substances (NPS) that have emerged across the world since the early 2000s. While NPS have been dominated in the news media by opioids, substituted cathinones remain an important class of drugs that have caused morbidity and mortality. Since 2011, the United States Federal government has used its emergency temporary scheduling powers to control 22 different substituted cathinones as Schedule I controlled substances.

Alpha-PiHP, also known as 4-methyl-1-phenyl-2-(pyrrolidin-1-yl)pentan-1-one or alpha-pyrrolidinoisohexanophenone, is a substituted cathinone that is an isomer of another NPS – alpha-PHP or alpha-pyrrolidinohexanophenone. Alpha-PiHP is also a positional isomer of the prescription medication pyrovalerone, a norepinephrine-dopamine reuptake inhibiting drug that is used for the treatment of chronic fatigue and as an appetite suppressant. Alpha-PiHP was first reported as being a drug sold on the illicit drug market in 2016 by the National Forensic Laboratory of Slovenia when it was detected in an off-white colored powder. In the United States in 2018, the Center for Forensic Science Research and Education (CFSRE) reported the detection of the substance in a white solid material via the Department of Homeland Security. Since 2021, NPS Discovery has included alpha-PiHP in its forensic toxicology laboratory NPS scope recommendations as either a tier one (strongly recommend) or tier two (recommend) listing.

Due to reports of the recent increase of alpha-PiHP in the US, in December 2022, we added the substance to our scope of comprehensive testing in postmortem blood samples. The objective of this study is to describe the prevalence of alpha-PiHP in our toxicology casework for 01/01/2023 – 06/01/2023, along with the other substances simultaneously detected. Cause of death determination, if available, as certified by the medical examiner was included.

Blood specimens were drawn by the medical examiner at autopsy, collected in vials containing sodium fluoride (NaF) as a preservative, and sent to the laboratory for toxicological analyses. Screening procedures included a comprehensive screen for drugs of abuse, prescription drugs, over-the-counter drugs, and NPS by liquid chromatography with quadrupole time of flight mass spectrometry (LC-QToF-MS) and volatile analysis by head space gas chromatography with flame ionization detection (HS-GC-FID). Qualitative identification of alpha-PiHP was undertaken by a protein precipitation extraction with acetonitrile followed by LC-QToF-MS detection. Alpha-PiHP was able to be chromatographically separated from alpha-PHP for analytical identification purposes. Limit of detection for alpha-PiHP was 5 ng/mL. Quantification was not completed. All other reported confirmatory analyses were completed by liquid chromatography with triple quadrupole mass spectrometry (LC-MS/MS) or gas chromatography with mass spectrometry (GC-MS).

For 01/01/2023 – 06/01/2023, we identified the presence of alpha-PiHP in 7 different postmortem blood samples. All detections were located within the state of Florida. The substance was not detected in any other location in which we do casework. It was detected as the sole substance in 2 cases and was detected alongside fentanyl in 3 cases and dimethylpentylone/pentylone in 2 cases. In the 3 cases where cause of death certification was available, alpha-PiHP was implicated in all as the drug of toxicological interest.

Alpha-PiHP was detected in 7 cases in our laboratory from 01/01/2023 – 06/01/2023. As with any NPS, it is prudent that a forensic toxicology laboratory assesses drug trends and prevalence for the locations which submit work to them and adapt their scopes of testing. If a laboratory is not screening for Alpha-PiHP, it is possible to be missing potentially positive casework.

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P055

Prevalence of tramadol and its metabolites in the Orange County DUID population over a 6-year period

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Abstract

Introduction: Driving under the influence of drugs (DUID) investigations have continued to gain interest within the field of forensic toxicology with increased awareness of prescription drug impairment, larger combinations of drug coingestion incidence, and increased technology for detecting multiple drugs in a single assay.

Tramadol (Ultram) is typically prescribed to treat pain, acting as an analgesic on the μ-opioid receptors. It is rarely found in street distribution as a drug of abuse, being detected in 198 (0.47%) of the solid dose cases in Orange County over the 6-year study period. Its interpretation of drug effects is compounded with the o-demethylated metabolite being an active metabolite at approximately twice the potency of the parent drug. Tramadol has also been reported to have some atypical narcotic effects, possibly causing elevation of pulse and blood pressure.

Objectives: This presentation will examine the presence of tramadol and its metabolites in the Orange County DUID population over a 6-year period (2017-2022). It will also review other drugs detected in these cases and review symptomology associated with tramadol use.

Methods: Blood samples submitted to the Orange County Crime Laboratory from January 1, 2017- December 31, 2022, were evaluated. Samples screened prior to August 2018 were screened for drugs using a 7-panel Immunoassay screen, not including tramadol. Samples screened after August 2018 were screened using a 4-panel Immunoassay in conjunction with a QTOF drug screen which included tramadol and the two metabolites. All samples included in this study underwent quantitative analysis for tramadol, N-desmethyltramadol, and/or o-desmethyltramadol by LC-MS/MS with limits of quantitation of 25 ng/mL, 25 ng/mL, and 50 ng/mL respectively.

Results: Over the 6-year span of this study, 149 (0.34%) DUID cases were submitted that subsequently had quantifiable levels of tramadol, N-Desmethyltramadol, and/or o-desmethyltramadol. The average tramadol concentration was 336.24 ng/mL (median 157.98 ng/mL), N-Desmethyltramadol average concentration 178.23 ng/mL (median 99.76 ng/mL), and o-desmethyltramadol average concentration 199.57 ng/mL (median 125.17 ng/mL). The year 2017 had the overall highest concentrations averaging 582.91 ng/mL (median 250.13 ng/mL), 341.25 ng/mL (median 168.9 ng/mL), and 478.17 ng/mL (median 247.79) for tramadol, N-desmethyltramadol, and/or o-desmethyltramadol respectively. The overall lowest concentrations were observed in 2021, averaging 182.49 ng/mL (median 97.42 ng/mL), 70.28 ng/mL (median 50.39 ng/mL), and 73.07 ng/mL (median 71.05 ng/mL) for tramadol, N-desmethyltramadol, and/or o-desmethyltramadol respectively.

All but 3 cases (97.9%) were poly-drug cases, with CNS depressants (89.3%) being the most common drug category seen in combination. Individually, alcohol coingestion accounted for 53% of the cases, other CNS depressants were detected in 61.7% of cases, followed by narcotic analgesics (32.9%), CNS stimulants (23.5%), and cannabis (22.8%)

The 3 cases where tramadol was the only drug detected were all from 2017 and all 3 cases had tramadol concentrations consistent with toxic levels. Police reports were obtained for these cases, and the observed effects will be discussed.

Discussion: Tramadol is a DUID drug of interest due to its atypical symptomology as a narcotic analgesic and toxicologically significant active metabolite. Tramadol has been listed as a Tier 1 drug on the National Safety Council's recommendations for drug testing in drivers and is a substance that would most likely be missed by traditional Immunoassay only screen procedures if not using a tramadol specific assay. This study is meant to aid in increasing the body of knowledge on tramadol as it relates to DUID investigations.

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P056

Sevoflurane and Ethanol - How Hospital Treatment for an Injury Can Cause Big Problems in a Standard Alcohol Case

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Abstract

Introduction: Sevoflurane belongs to a group of medicines known as general anesthetics. It is administered to cause loss of consciousness before and during surgery and is often preferred over other anesthetics due to its rapid induction and recovery characteristics. Like ethanol, sevoflurane is a volatile compound that can be detected by headspace gas chromatography (HSGC). In late 2019 and early 2020, two cases were submitted to the laboratory for volatiles testing in which the suspect had been given sevoflurane during the course of medical treatment. Volatiles testing is performed at DFS using dual column HSGC, with one column used for quantitation and the other used for confirmation. Sevoflurane interferes with our ability to report ethanol concentrations because it coelutes with ethanol on the column used for confirmation.

Objectives: The objective of this research was to develop a method which could be used in combination with HSGC to allow us to confirm and quantitate ethanol in the presence of sevoflurane. One of the cases affected involved a driver that caused the death of two people and serious injury to two others. Due to the severity of the incident, it was important to the prosecution that we be able to report an ethanol concentration.

Methods: Blood samples were prepared using a modified version of the DFS trace evidence unit procedure used to analyze fire debris and ignitable liquids. The method was validated to ANSI/ASB standard 036, Standard Practices for Method Validation in Forensic Toxicology (1st edition, 2019). Studies included carryover, interferences, limit of detection, and stability. Extraction of volatiles from blood was accomplished by passive headspace adsorption using activated carbon strips (c-strips). A c-strip was placed in the headspace of each tube, the tubes were heated for approximately 4 hours, and the c-strips were desorbed with a small volume of carbon disulfide (CS2). The CS2 was transferred to autosampler vials and the samples were analyzed by Gas Chromatography Mass Spectrometry (GCMS). Samples were analyzed in full scan mode on an Agilent 7890B GC/5977B MS equipped with an HP-5ms Ultra Inert GC Column (30 m length, 0.25 mm inner diameter, 0.25 µm film thickness). The oven temperature and other instrument parameters were as follows:

	Rate °C/min	Value °C	Hold Time Min	Run Time Min
(Initial)		40	4	4
Ramp 1	30	130	0	7

Inlet Temperature °C	Column Flow L/min	Transfer Line Temperature °C	MS Source Temperature °C	MS Quadrupole Temperature °C
250	0.8	250	230	150

Results: No carryover was observed after the high concentration control (methanol, ethanol, acetone, isopropanol at approximately 0.500 g/100mL and sevoflurane at 3.0 ug/mL) and no interferences were detected with other commonly encountered analytes (methanol, ethanol, sevoflurane, isopropanol, acetone, and t-butanol).

The limits of detection were determined to be 0.010 g/100mL for ethanol, isopropanol, and acetone; 0.3076 µg/mL for sevoflurane; and 0.500 g/100mL for methanol. Methanol, ethanol, and sevoflurane are stable only on the day of sample preparation; t-butanol up to one day after sample preparation; and isopropanol and acetone up to two days after sample

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preparation.

Discussion: This validation demonstrated the qualitative identification of methanol, ethanol, sevoflurane, isopropanol, and acetone by GCMS. The quantitative ethanol concentration was reported from the HSGC method, where a full calibration curve is run. The GCMS method was then used as the secondary method for confirmation. The work for this study took place between 4/8/2020 and 5/26/2020. The method was approved for casework and reports were written for both cases on 7/8/2020. Should we encounter cases in which ethanol and sevoflurane are present in the future, the turnaround time for results will be much quicker.

P057

Chemicals in Urine Can Reduce Glucuronide Hydrolysis Efficiency Causing False Negatives for Drug of Abuse Analysis

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Abstract

Introduction: Urine drug testing is one of the most common practices for monitoring the use of prescribed or illicit medications. Testing is typically performed by a preliminary screening assay, such as immunoassay, followed by a confirmatory assay such as liquid chromatography coupled with mass spectrometry (LC-MS/MS). β-glucuronidases hydrolyze glucuronidated compounds from phase II metabolites present in urine which simplifies parent analyte detection using LC-MS/MS. This study shows that endogenous chemicals in clinical samples can reduce enzyme performance compared to control samples.

Objectives: Present data to show that endogenous chemicals in clinical samples could reduce enzyme hydrolysis compared to control samples. Not all commercially available recombinant enzymes are inhibited by these chemicals equally. Additionally, using an inadequate amount of enzyme could result in low hydrolysis for drugs of abuse which can potentially cause false negatives.

Methods: Drug standards were from Cerilliant. All reagents were purchased from MilliporeSigma or Fisher Scientific. Opioid-positive urine specimens were obtained from a national testing laboratory and had no identifying information. Drug free human urine control was from UTAK and was fortified with glucuronidated drugs of abuse. Two commercially available β-glucuronidases from two different manufacturers are both advertised as room temperature hydrolysis. One is IMCSzyme RT from IMCS the other is "Enzyme A". Control and patient specimens were buffered and hydrolyzed with two commercial glucuronidases for 15-minutes at room temperature. The two β-glucuronidases were compared by two different methods. First method, ninety-six patent samples were hydrolyzed using an equivalent protein amounts of β-glucuronidases, measured by Bradford. Second method tested patient samples with a range of β-glucuronidase amounts that are below, at and over manufacturer recommendations.

Sample clean-up was performed by eluting hydrolyzed urine samples through β-Gone plus plates from Phenomenex and diluted with water. Sample were 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: Ninety-six patient samples were hydrolyzed with equivalent protein amounts by two different β-glucuronidases. Samples were considered positive if opioid recovery was ≥ 25 ng/mL. Out of 96 patient samples analyzed, 50 results disagreed between IMCSzyme RT and Enzyme A where they were reported above the cutoff specification when hydrolyzed with IMCSzyme RT and below the cutoff specification when hydrolyzed with Enzyme A. Missing such thresholds resulted in 50 results being potentially reported as false negatives.

Four patient samples and a fortified control sample were hydrolyzed with a range of β-glucuronidase concentrations (0-100 µg of one β-glucuronidase per reaction and 0-200 µg of the second per reaction). Hydrolysis was reported as % hydrolysis and considered complete when hydrolysis reached ≥ 80%. Complete hydrolysis was confirmed based on ≤ 20% glucuronide remaining in the sample. Two out of four patient samples required more enzyme to complete hydrolysis than the control sample, indicating that these samples contain endogenous chemicals that reduced enzyme hydrolysis of morphine and oxymorphone glucuronide. Endogenous chemicals in urine that reduced enzyme activity include urea, ascorbic acid and flavonoids.

Discussion: Some patient samples contain different amounts of endogenous chemicals that reduce enzyme activities compared to control samples and not all enzymes are affected equally by these chemicals. Using an inadequate amount of enzyme could result in low rates of hydrolysis for drugs of abuse, and ultimately, false negatives for patient samples.

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P058

Benzonatate: Cures acute cough, causes analytical headache.

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Abstract

Introduction: Benzonatate (Tessalon®) was approved in 1958 as a peripherally acting antitussive that is structurally related to the local anesthetics (LA) procaine and tetracaine. It is currently available in 100 mg Perles or 200 mg capsules, for patients 10 years of age and up, with a maximum listed daily dose of 600 mg/day. In 2010, benzonatate received attention when it was assigned a black-box warning because the capsules resemble candy, which may be desirable to young children. While this drug could be an alternative to opioid-containing cough medicines, its impact on public health and safety should be evaluated based on its pharmacokinetics, potential for toxicity, and analytical detectability in a forensic laboratory setting.

Objectives: In several of the published over-ingestion cases, as well as those observed in our office, a small handful of Tessalon® pills was enough to cause seizures, a cardiac event, and death. This presentation will spread awareness to the toxicological community about the dangers of over-ingestion of benzonatate and how to interpret the analytical data resulting from benzonatate exposure. A case from 2022 at the North Carolina Office of the Chief Medical Examiner (NC OCME) involving a benzonatate prescription will be highlighted to demonstrate the analytical advancements in our laboratory used to help the medical examiner understand the complexities of this analyte of interest.

Results: Benzonatate exists as a mixture of compounds and is rapidly hydrolyzed by plasma butyrylcholinesterase (BChE) to the major metabolite 4-butylaminobenzoic acid (BABA) along with the corresponding polyethylene glycol monomethyl ethers. The North Carolina Office of the Chief Medical Examiner (NC OCME) discovered several benzonatate-related cases based on the recognizable benzonatate-related peak pattern. The series of polyethoxy compounds (e.g. n=9 identified by the NIST mass spectral database as 2-[2-[2-[2-[2-[2-[2-[2-(2-Methoxyethoxy)ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy] ethanol) and BABA (identified by comparison to a USP standard) were detected using a GC-MS/FID acidic/neutral screen, and a phthalate peak of unknown origin appeared in a GC-MS/NPD basic-drug screen. In the past, parent benzonatate was only detected via screening in one of the overdose cases at NC OCME. It appeared as a late-eluting compound after trazodone in the base screen and was quantitated by a subcontracting laboratory using traditional HPLC methodology. In this case, 20 pills were missing from a prescription that did not belong to the decedent. In contrast, the subcontracting laboratory did not detect benzonatate in another suicidal overdose with a note and several empty bottles of medication found at the scene, including benzonatate, using their traditional methodology; however, the remaining toxicology did not support a drug cause of death by other substances. The lack of detectable parent compound in other decedents was explained by rapid hydrolysis, a common trait of ester-based local anesthetics.

The 2022 case involved a 16-year-old decedent, with an appropriately empty prescription for 200 mg benzonatate capsules, whose death was certified as undetermined/unknown by the pathologist. The NC OCME laboratory qualitatively reported the BABA metabolite in postmortem femoral blood and urine on the final report; however, lack of scene findings did not allow for a drug-related cause of death. Once benzonatate solution in ethanol (Cayman Chemical Item No. 23936) was obtained later, the parent mixture of analytes was detected in the femoral blood of the decedent using a Thermo Scientific Orbitrap with parallel reaction monitoring (PRM).

Discussion: Benzonatate remains an analytically problematic pharmaceutical for postmortem samples, resulting in an under-reporting of its presence in forensic laboratory reports. Death scene details obtained through investigation by the medical examiner help to evaluate the possibility of benzonatate over-ingestion.

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P059

The Rising Prevalence of Fentanyl With Methamphetamine in DUID Cases

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Abstract

Introduction: The presence of multiple drugs can affect a person's ability to multitask and may display different impairments in a driving while under the influence of drugs (DUID) case. Historically, the concurrent misuse of opioids and psychomotor stimulants with abuse potential has been observed frequently together. Specifically, methamphetamine is a highly addictive stimulant that acts on the central nervous system and can affect a person's driving ability. While fentanyl as a synthetic narcotic analgesic can also cause the impairment of psychomotor performance and is now deemed as the next synthetic opioid crisis that rose across the United States. Alarmingly, fentanyl in combination with methamphetamine is being seen as one of the deadly pairings with symptomology that are not typically expected in a DUID case. The study focused on the impairment of fentanyl and methamphetamine, as they are now ranks as one of the top three drug combinations in DUID cases in Orange County, California.

Objective: To provide concentration and statistical data of DUID cases with fentanyl and methamphetamine from 2018 to 2022 in Orange County, CA.

Method: Screening: Methamphetamine and fentanyl were screened by a Shimadzu Nexera HPLC with an AB Sciex x500R QTOF with Phenomenex Kinetex® Phenyl Hexyl column, 2.6 µm, 50 x 4.6 mm with Phenyl Guard column. The limit of detection for fentanyl is 0.5 ng/mL, in ESI+ mode.

Quantitation: After a liquid-liquid extraction, the concentration of methamphetamine is determined using Agilent 7890B GC, 5977A MS with HP-1, 25 m, 0.20 mm, 0.33 µm column. Qualifier ions for methamphetamine are 118 and 154, with the quantitation ratio at 154/158 with dwell time at 15 ms. Calibration curve starting from 20 ng/mL to 4000 ng/mL, with controls at 240 ng/mL and 2400 ng/mL.

For fentanyl, the extraction procedure included a protein precipitation with cold acetonitrile and a filtration step with DPX Wax-S tips. Quantitative analysis was performed using Waters XeVo TQ-S with Acquity UPLC with Phenomenex Kinetex® column 1.7 µm Biphenyl Å, 2.1 x 100 mm. The calibration curve starts at 0.5 ng/mL to 32 ng/mL along with controls at 1.5 to 15 ng/mL.

Results: Over the course of 5 years, from 2018 to 2022, detection of fentanyl increased from 159 cases to 1341 cases per year, respectively. In conjunction, an increase was seen with fentanyl and methamphetamine in combination, with detection increasing from 31 cases in 2018 to 314 cases in 2022, a 1013% increase. Concentration of fentanyl is at an unpredictable pattern, overlapping with the postmortem concentration range. For these poly-drug cases, fentanyl concentrations were detected starting from 0.53 ng/mL up to 243.64 ng/mL and methamphetamine at a range from 27.3 ng/mL to 7777 ng/mL. Additional compounds that were frequently reported with the pairing were morphine, THC, and alprazolam. The drug combination of fentanyl, methamphetamine, and morphine was in 71% of cases in 2018, but significantly decreased to 7% in 2021 and 2% in 2022. In comparison, the detection of cannabis with fentanyl and methamphetamine fluctuated from 29% in 2018 to 15% in 2020 and resurfaced in 2022 with 22%.

Conclusion: The increased risk of car accidents from impairment of psychomotor performance correlates with the addition in the present of compounds found in DUID cases. The drug combination of an opioid and a stimulant would require knowledge in the anticipated symptomology and how to carefully make a comprehensive deduction with the totality of the case information. Further training and testing are advised to prepare forensic toxicologists for the anticipated drug trends that are changing rapidly.

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P060

Using a Virtual Liquid Chromatography Tool to Develop Methods for Novel Psychoactive Substances

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Abstract

Introduction: Novel psychoactive substances (NPS) have created a challenge for toxicology laboratories. New NPS are constantly disappearing as fast as they emerge, making it difficult to stay on top of which compounds are necessary to add to laboratory testing scopes. The development and optimization of liquid chromatography (LC) separations is time consuming and costly, often requiring several steps including literature research, column selection, method scouting, method development, and method optimization. To alleviate the burden of sacrificing instrument-uptime, labor and materials, an instrument-free software modeling tool was developed to include a comprehensive drugs of abuse (DoA) library. This online tool allows users to obtain optimized separations while maintaining critical pair resolution by adjusting parameters such as column dimension, mobile phase, gradient programs, and more for almost 300 compounds including the 38 newly added NPS drugs.

Objectives: The primary objective of this study is to use a chromatographic modeling tool to develop effective LC-MS/MS methods for various NPS compounds including synthetic opioids, designer benzodiazepines, synthetic cathinones, synthetic cannabinoids, and toxic adulterants.

Method: The NPS library utilized the same design space as the existing DoA library. Retention times were collected using method conditions consisting of a fast (5 minute) and slow (15 minute) gradient, 30°C/60°C temperature points, and ACN/MeOH mobile phases on Raptor Biphenyl and Raptor C18 columns in a 50 x 2.1, 2.7 µm dimension. The 38 NPS compounds were divided into three small groups to account for the separation of isobars and to generate the optimal points per peak for instrument analysis. A set of 8 compounds, referred to as "meld compounds", were then added to each group. These meld compounds spanned the chromatographic space and were used to verify instrument performance from injection to injection. Data was collected and input into the platform. Results of retention times between experimental and modeled data were compared. To verify the ability of the modeler to develop methods for NPS, three methods were developed and optimized using the chromatogram modeler for the following NPS subclasses: 1) synthetic opioids and toxic adulterants 2) designer benzodiazepines 3) stimulants and synthetic cannabinoids. All methods utilized a Raptor Biphenyl 100 x 2.1, 2.7 µm column with a MPA of water and MPB of methanol, both acidified with 0.1% formic acid. The flow rate was 0.6 mL/min and the column temperature was 40°C. The developed methods were transferred to an LC-MS/MS system and the experimental results were compared with the modeler.

Results: The online chromatogram modeling tool successfully developed methods for NPS compounds. Developing the methods using the virtual chromatography tool was completed in under ten minutes per method. The acceptance criteria for retention time agreement between experimental and modeled values was set at +/- 15 seconds, chosen to represent a typical MRM window. All analytes in all three methods fell within this window, as well as maintaining elution order and resolution. For example, Isotonitazene had a predicted retention time of 2.86 minutes and an experimental retention time 2.75 minutes, for a difference of 6.6 seconds. Eutylone had a predicted retention time of 4.42 minutes and an experimental retention time of 4.18 minutes, for a difference of 14.4 seconds. Based on the acceptance criteria as defined, each NPS method was successfully transferred from the virtual model to an LC-MS/MS instrument.

Conclusion: As NPS continue to proliferate the illicit drug market, the burden of adding these compounds to laboratory testing scopes becomes the obligation of LC method developers. Utilizing tools such as a virtual chromatography modeler can help method developers deal with the challenges these emerging compounds present.

POSTER

Benefits and Impact of the Professional Mentoring Program to SOFT

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Abstract

Introduction: A primary goal of the SOFT Professional Mentoring Program (PMP) is to develop and nurture future leaders of the organization. The program was designed to provide a framework to set measurable goals between paired partners. To assist the relationship, our program is driven to meet the expectations of the mentees and mentors while creating space for the pairing to be independently guided with developmental tools and resources. Each program subcommittee incorporates participant feedback to continually improve the program experience and nurture SOFT leaders at all professional stages. Participant surveys assess committee-defined mentoring benefits, track progress between pairs, establish measurable outcomes, and target program improvements.

Objective: To share program outcomes of mentor/mentee self-reports and emphasize the impact of the PMP within SOFT.

Methods: Feedback on participant achievements (goals assessment), and program structure/content (program value assessment) were collected via participant surveys in 2020, 2021 and 2022. Similar questionnaires were provided all years; however, the 2021/2022 assessment limited open-ended questions to facilitate data analysis. In addition to one-on-one mentoring sessions, the 2022 program offered other activities based on the 2021 participants' feedback, including a kick-off event, webinars, resources emails and a mentoring reception at SOFT.

Results: Since the inaugural year, the program has met the participants' original expectations, and exceeded them in all years: 88% in 2020, 90% in 2021 and 92% in 2022. The breakdown of program participation for 2020-2022 is shown in the table below. For professional goals, the program has shown an impact mainly in 3 areas: career advancement, development of interpersonal/leadership skills and SOFT engagement. According to survey data, the most significant outcome was SOFT engagement in 2020 (54.3%), career advancement in 2021 (63.9%), and career advancement and development of interpersonal/leadership skills in 2022 (52.3% each). In all 3 years, participants ranked among the top 3 benefits from the program: the transfer of knowledge (>75%); expanding their professional network in 2020 (83%) and 2022 (94%), and encouraging voices in 2021 (88%) and 2022 (77%). Other highly ranked benefits (>75%) were feedback in 2020, growth of talent to innovate the field in 2021, and leadership in 2022. Among activities organized in 2022, the most valued (70-87% high or moderate value) were an invited speaker webinar on Impostor Syndrome, kick-off event, TED talk and discussion, and monthly mentoring resource emails.

Program Year	Participants	New Pairs	Continuing Pairs
2020	74	41	n/a
2021	84	45	6
2022	95	41	15

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Discussion/Conclusion: Founded in 2019, the SOFT mentoring program continues to grow and improve in response to crucial participant feedback. Respondent data supports the professional and developmental value of a structured mentorship program to the SOFT organization. Both participation and increased positive outcomes were reported from 2020 through 2022. The program was designed to provide a setting to establish professional relationships guided by agreed-upon goals from strategically paired members of the society. Rather than traditional one-way mentoring, the participants are encouraged to initially spend time defining a mutually beneficial vision to work towards during program participation that will optimally develop into more collaborative relationships, leadership development and legacy-building transfer of knowledge within SOFT.

P061

Development and Validation of a Comprehensive Dilute-and-Shoot LC-MS/MS Method: Qualitative Confirmation of 113 Drugs in Urine

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Abstract

Introduction: Although comprehensive drug toxicology methods are critical for emergency care, extensive extraction protocols, long analytical run times, and labor-intensive review processes make them difficult to operationalize in high-throughput laboratories. Improvements in liquid chromatography-tandem mass spectrometry (LC-MS/MS) sensitivity, scan speed, and fast polarity switching during multiple-reaction-monitoring (MRM), combined with room-temperature enzymatic hydrolysis, enable analysis of large drug panels with automated sample extraction and without the need for incubation.

Objectives: Develop and validate a comprehensive urine panel utilizing automated dilute-and-shoot extraction with room temperature hydrolysis, and automated LC-MS/MS data processing.

Methods: Negative urine was collected and fortified with 113 drugs belonging to 12 different categories: anticonvulsants, antidepressants, antipsychotics, barbiturates, benzodiazepines, local anesthetics, opioids, over-the-counters, muscle relaxers, sedatives/dissociative anesthetics, stimulants, and 11-nor-9-carboxy-delta-9-tetrahydrocannabinol (THCCOOH). Compounds were from Cerilliant (Round Rock, TX), Cayman Chemical Company (Ann Arbor, MI), or Toronto Research Chemicals (North York, ON, Canada). Solvents were from Fisher Scientific (Hampton, NH) or Millipore Sigma (St. Louis, MO).

A linear 3-point calibration model was utilized to establish qualitative cutoffs. The dilute-and-shoot method involved hydrolyzing urine specimens at room temperature using diluted B-One enzyme (Kura Biotech, Los Angeles, CA) and fortified with a solution containing 13 deuterated compounds as internal standards (IS). Samples were subsequently diluted with a mix of mobile phase A (5 mM ammonium formate and 0.1% formic acid in water) and acetonitrile. Analytes were resolved using an 11.4 minute gradient of mobile phase A to mobile phase B (0.1% formic acid in 75:25 methanol:acetonitrile) with an Exion LC system and a Kinetex 2.6 µm Biphenyl 100 Å 50x4.6 mm column (Phenomenex, Torrance, CA) coupled to SCIEX 5500+ MS (Framingham, MA). All analytes were monitored using 2 MRMs and 1 ion ratio for acceptance, except for ibuprofen (1 MRM). One MRM was used for each IS, which were chosen based on retention times to avoid overlap with commonly highly concentrated analytes. Ibuprofen, ritalinic acid, and THCCOOH had their own matched ISs to compensate for matrix effects observed in prevalidation studies. Automated data processing involved Ascent software (Indigo Bioautomation, Carmel, IN). Validation studies were performed per ANSI/ASB standards.

Results: Limits of quantitation (LOQs) ranged from 5 ng/mL to 1,500 ng/mL and upper limits of linearity (ULOLs) were 6×LOQs. Within run precision ranged from 1.4% to 8.3% (n=5), and between run precision from 1.8% to 9.7% (n=25). No matrix effect was observed; all drugs fortified at 2 concentrations in 15 different donor specimens demonstrated peak areas within ± 25% of fortified mobile phase A samples. No carryover was observed with drug levels up to 1 mg/mL. Urine specimens were stable for 14 days at room temperature (15 to 30°C), 21 days refrigerated (2 to 8°C), and 30 days frozen (≤ -10°C). Hydrolysis efficiency was established in a mix of 15,000 ng/mL codeine-6β-D-glucuronide (92% hydrolyzed), 10,332 ng/mL oxazepam glucuronide (84% hydrolyzed), and 2,461 ng/mL naloxone-3β-D-glucuronide (90% hydrolyzed). No clinically significant interferences from the 113 in-panel analytes or 51 related substances were observed within their normal ranges of concentrations in patient specimens. Some analyte suppression at extremely high interference concentrations was observed and delta8- and delta9-THCCOOH isomers were not resolved.

Discussion: A high-throughput LC-MS/MS method was developed and validated for 113 analytes in urine utilizing automated specimen dilution. For emergency medicine, suppression from high-concentration interferences should be monitored and THCCOOH isomer separation is recommended if needed for confirmation. The assay provides a definitive and comprehensive qualitative assessment for these drugs, their presence indicating substance misuse and toxicity when nonprescribed, their absence indicating poor adherence or diversion when prescribed by a healthcare provider.

POSTER

P062

Simplified Workflow for Whole Blood Testing in Medicolegal Death and Impaired Driving Cases

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Abstract

Introduction: In response to the National Research Council report of 2009 that called for standardized methods and approaches in forensic science, the Academy Standards Board (ASB) recently adopted new standards to define the analytical scope and sensitivity requirements for medicolegal death and driving impairment cases (1,2). Forensic laboratories struggle to keep pace with these new standards because they often lack adequate technology and personnel required for method development and implementation. The goal of this study was to simplify forensic testing workflows for both medicolegal and impairment case work to reduce operational burdens and to help laboratories sustain toxicology testing.

Objective: This study validates a single liquid chromatography tandem mass spectrometry (LC-MS/MS) method for detection and quantification of 123 high priority drug targets and downstream metabolites characterized as opioids, stimulants, benzodiazepines, cannabinoids, and other drug classes listed in ASB standards for whole blood testing (3,4). This analytical procedure is suitable for use in medicolegal and driving impairment cases.

Methods: Commercially available ToxBox® (PinPoint Testing LLC, Little Rock, AR) test kits with premanufactured standards, quality control materials and internal standards in a "ready-to-use" 96-well plate format for high-throughput testing applications were used. Samples were processed using the ToxBox® equipped with supported liquid extraction for sample clean-up. Chromatographic separations using identical mobile phases and gradients were harmonized across two orthogonal stationary phases (C18 and phenyl-hexyl, Phenomenex, Torrance, CA) to facilitate concordance testing. Method performance indicators followed ASB accreditation requirements: accuracy, precision, measurement uncertainty, calibration model, reportable range, sensitivity, specificity, carryover, interference, ion suppression/enhancement, and analyte stability. (3,4).

Results: The method met all criteria for the new and forthcoming ASB experimental protocols, testing and reporting requirements for LC-MS/MS analysis in forensic laboratories (1,2).

Discussion: This "hands-free" testing technology creates a more sustainable testing environment by decreasing both administrative and technical burdens. For example, smaller chemical inventories need to be maintained with fewer methods, and with a single clean-up procedure many different classes of drugs can be assayed simultaneously with entry level equipment to meet new ASB reporting requirements. This study shows that the analytical procedure and new technology presented as part of this study are suitable for drug facilitate crime investigations.

POSTER

References

1. AAFS Standards Board. Standard Practices for Method Validation in Forensic Toxicology, ANSI/ASB Standard 036; Colorado Springs, CO, 2021. 24.

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3. AAFS Standards Board. Standard for the Analytical Scope and Sensitivity of Forensic Toxicological Testing of Blood in Medicolegal Death Investigations; ANSI/ASB Standard 119; Colorado Springs, CO, 2021

4. AAFS Standards Board. Standard for the Analytical Scope and Sensitivity of Forensic Toxicological Testing of Blood in Impaired Driving Investigations, ANSI/ASB Standard 120; Colorado Springs, CO, 2021

P063

Streamlined LC-MS/MS Workflows for Drug Facilitated Crime Investigations

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Abstract

Introduction: ToxBox® forensic test kits provide low-level, quantitative, high-throughput testing by liquid-chromatography tandem mass spectrometry (LC-MS/MS). Traditional ToxBox® kits speed sample analysis with analytical and internal standards at precise concentrations in a *ready-to-go* format where only blank matrix, enzyme/buffers and casework specimens are added. New ToxBox® kits with suspended-state technology incorporate standards, blank matrix, enzyme/buffers in solid-state, with air gaps to stabilize components for long-term storage. This unique design streamlines validation and increases efficiency because only casework specimens need to be added. These new kits are available for drug facilitated crime investigations (DFCI) (1); however, they have not been validated to ISO17025 accreditation standards.

Objective: Validate an LC-MS/MS simplified urine drug testing workflow for DFCI (1,2) using traditional ToxBox® kits, then evaluate new suspended-state technology for DFCI casework.

Methods: Drug residue in the traditional ToxBox® was matrix-matched by reconstituting with 100 µL of blank urine. Calibration (0.1 ng/mL to 100,000 ng/mL) and QC (0.3 ng/mL to 90,000 ng/mL) concentrations met sensitivity requirements established for DFCI (1). Samples were processed with the addition of β-glucuronidase and concentrated by liquid extraction using the ToxBox® kit protocol. Chromatographic separations were validated for concordance testing using two orthogonal C18 and phenyl-hexyl stationary phases. Chromatographic separations were separately validated using identical mobile phases and gradients. MS/MS parameters maximized analytical measurement ranges on an Agilent 6420 LC-MS/MS. Two m/z transitions (quantitative and qualitative) that minimized potential interferences for each analyte were chosen for confirmational analysis.

Results: The LC-MS/MS method was validated for 60 analytes from multiple drug classes encompassing analgesics, sedatives, illicit and prescribed drugs, and their metabolites using traditional ToxBox® kits. Within the validated reportable range, recovery (% bias), within- and between-run precision (%CV), and measurement uncertainty was < 20% for all analytes. Except for lorazepam, no difference was observed between the two stationary phases. All other analytes met performance specifications and ASB reporting requirements. The clonazepam internal standard interfered with the confirmation ion of lorazepam when the phenyl-hexyl column was used. The C18 column provided adequate resolution to confirm lorazepam above 10 ng/mL (ASB defined cut-off is 5 ng/mL). More sensitive LC-MS/MS instrumentation or a larger sample volume would be required to meet DFCI reporting requirements for lorazepam.

Next, the validated procedure was used to evaluate suspended-state technology for urine toxicology. No differences were observed when results were compared. The %bias and %CV were < 20% for all analytes.

Conclusions: This study demonstrates a new LC-MS/MS testing procedure for commercially available ToxBox® test kits. The 96-well plate format supports high-throughput testing for DFCI. No differences were observed between the traditional and new kits that incorporated suspended-state technology. Both kit designs meet accreditation requirements for all analytes tested; however, lorazepam could only be validated to 10 ng/mL using 100 µL sample and entry level LC-MS/MS equipment. This new LC-MS/MS testing procedure is suitable for DFCI casework.

References

1. ANSI/ASB Standard 121; Colorado Springs, CO, 2021
2. ANSI/ASB Standard 036; Colorado Springs, CO, 2021

POSTER

P065

A Comparison of the Performance of LC-MS/MS Analytical Methods for Workplace Drugs of Abuse Testing Using Zero-Grade Air and Nitrogen Gas

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Abstract

Introduction: With the continual growth and addition of Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS) instruments to our laboratory, addressing the increasing consumption of nitrogen gas became a necessity. In order to support round-the-clock operation of 37 Sciex mass spectrometers nearly seven days a week, our 6,000 gallon supply of nitrogen needed to be replenished every 5 days; any unforeseen delay in delivery could result in a potential laboratory shutdown. Use of zero-grade air for source exhaust and Gases 1 and 2, which Sciex recommends, would reduce laboratory nitrogen consumption approximately 70%, extending the service of the 6,000 gallon nitrogen supply to 17 days. Prior to conversion, it was necessary to evaluate the impact of zero-grade air on analytical methods that were validated using nitrogen gas.

Objective: Determine any differences in ionization or performance for Sciex LCMS/MS instrumentation while operating with zero-grade air compared to nitrogen for the source gas in the analysis of workplace drugs of abuse confirmation.

Methods: Abbreviated validation sample batches were prepared to evaluate the linearity and investigate potential interference for each assay. Linearity studies were comprised of standard replicates at concentrations equal to the lowest level of quantitation, cutoff calibrator, and upper limit of linearity for all analytes. Method interferences were examined using matrix-matched negative samples and samples formulated at 40% of cutoff concentration that were spiked with over-the-counter, prescription, and illicit drugs. Batches were initially analyzed using validated instrument methods with nitrogen gas for source exhaust and Gases 1 and 2. Gas lines were subsequently switched to zero-grade air and the samples were reanalyzed. All data was reviewed and results were compared for analyte and internal standard peak area counts and calculated concentration.

Results: All methods exhibited acceptable performance operating with zero-grade air for source exhaust and Gas 1 and Gas 2. Compared to analysis conducted with the use of nitrogen gas, the only method demonstrating pronounced change was the urine barbiturate assay, which operates in negative mode using atmospheric pressure chemical ionization (APCI), and displayed a twofold increase in ionization. Using electrospray ionization (ESI), a 10% decrease in ionization was observed in both urine and oral fluid Ethyl Glucuronide analysis. The oral fluid buprenorphine assay presented a 7% decrease in buprenorphine ionization and a 10% increase in norbuprenorphine ionization. In the oral fluid benzodiazepine assay, there was a 20% increase in Flurazepam ionization, and a 20% and 15% ionization decrease in Alprazolam and Triazolam, respectively.

Discussion: CRL completed a thorough evaluation of the impact of using zero-grade air in place of nitrogen gas for LC-MS/MS source exhaust and Gases 1 and 2 on all workplace drug testing confirmation methods. The comparison of validation data generated while operating with zero-grade air and nitrogen gas revealed few significant differences; however, the potential cost savings and increased business autonomy created by a 70% reduction in nitrogen consumption was

POSTER

P066

Trends in Suicides and Drug Involvement: Exploring the Impact of the Pandemic in the Forensic Toxicology Landscape

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Abstract

Introduction: Suicide is a global public health concern, and understanding its underlying factors and trends is crucial for effective strategies. Forensic toxicologists play a pivotal role in the investigation of suicide cases, particularly in assessing the presence of drugs involved in these incidents and their contribution to death. Furthermore, during periods of crisis such as the COVID-19 pandemic, it becomes even more critical to identify any shifts in suicide trends and drug preferences, as they may have far-reaching implications for both toxicological analysis and public service announcements.

Objectives: This study investigates the trends in suicides and the specific drugs used in suicide cases for Harris County, Texas, over the past five years, while also examining the potential impact of the COVID-19 pandemic on these patterns. Suicide is a complex phenomenon influenced by various factors, including mental health, societal stressors, and drug involvement. Understanding the interplay between suicide rates, drug utilization, and the effects of a global health crisis is crucial for forensic toxicologists in their pursuit of accurate toxicological assessments.

Methods: Using a comprehensive dataset of suicide cases from the Harris County Institute of Forensic Sciences, we conducted an in-depth analysis of suicide trends and the substances implicated in these acts. The study period spans from 2018 to 2022, encompassing both the pre-pandemic and pandemic eras. Statistical methods were employed to identify significant variations in suicide rates and assess the potential influence of the COVID-19 pandemic on drug-related suicides.

Results: Preliminary findings reveal relatively insignificant trends in suicides and drug utilization patterns. Although suicides, in general, have shown a steady increase with the onset of the pandemic, we observed minor changes in the prevalence and types of drugs associated with suicide cases throughout the study period. The majority of drugs implicated in suicides are readily available prescription medications or substances with easy access. Notably, leading into the pandemic and following, certain drugs/substances showed an increased presence in suicide cases, suggesting a potential shift in the substances used for self-harm in more recent history.

Discussion: The pandemic's widespread ramifications, including social isolation, economic strain, supply chain disruption, consumer access, and disruptions to mental health services, may have contributed to shifts in suicide trends and drug preferences. Not surprisingly, a large number of the drugs identified were prescription drugs in the classes of benzodiazepines, antidepressants, and antipsychotics. This may indicate a preceding struggle with underlying mental health issues. In contrast, common illicit substances (e.g. fentanyl, cocaine, and methamphetamine) responsible for death in many other cases were seen with lower prevalence. It is possible these drugs are under-represented as the necessary burden of proof to move the manner of death from accident to suicide is much higher. The toxicological interpretation of such substances in the absence of strong investigative evidence and medical/social history is difficult if not impossible. The lack of prevalence of these substances may then be a result of insufficient information found on scene or through investigation to be able to opine on intent. Furthermore, any increase in detection of various substances over the course of the time period could be a consequence of knowing to look for that substance in future cases and/or expanding scope based on earlier findings. One such example was seen through the increased detection of nitrates/nitrites in the later years of this study. By understanding the impact of the pandemic on suicide rates and drug preferences, toxicologists can adapt their analytical approaches and contribute to optimization of public health responses.

POSTER

P067

Evaluation of Sample Ionization Sources for the Characterization of Isomeric Fentanyl Analogs Utilizing Trapped Ion Mobility Spectrometry Time of Flight Mass Spectrometry (TIMS-ToF MS).

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Abstract

Introduction: Worldwide, especially in the United States, the public has been increasingly impacted by the utilization of fentanyl and the rise of its analogs in the early 2010s. Ever since, fentanyl has risen to become one of the leading causes of drug-related deaths in the United States. In recent years, it has consistently ranked among the top substances involved in fatal overdoses and fentanyl-related fatalities have surpassed deaths caused by other opioids, such as prescription painkillers and heroin.

Objective: This study aims to assess the utilization of various sample introduction ionization sources (direct infusion electrospray (ESI), nanoESI, direct analysis in real time (DART) coupled with trapped ion mobility spectrometry (TIMS) and mass spectrometry (MS). Employing these orthogonal techniques to comprehensively characterize fentanyl analogs by thoroughly investigating their isotopic distributions, characteristic ion mobility profiles, and fragmentation patterns.

Methods: A Fentanyl Analog Screening Kit was acquired from Cayman Chemicals, which contained over 200 synthetic opioids. Standards were prepared in methanol and diluted to a concentration of 1 ng/mL (1 ppb). Isomeric standards were organized into separate sample sets for their initial characterization. Experiments were performed on a custom built TIMS-q-ToF MS (Bruker Daltonics) equipped with a custom nESI, DART ionization source, or coupled to a Shimadzu Prominence HPLC (Shimadzu) for direct infusion experiments equipped with an Phenomenex Onyx monolithic C18 column (100 x 4.6 mm) from prior to ion mobility and MS analysis. Initial experiments were performed in positive ion mode using data dependent acquisition and data independent acquisition with resulting data processed using Data Analysis 5.2 software (Bruker Daltonics).

Preliminary Results/Discussion: 29 isomeric sets totaling 185 fentanyl analogs were characterized by examination of the 2D LC-MS and IMS-MS profiles which allowed for the identification based on their resulting isotopic pattern, retention time (direct infusion) and/or ion mobility profiles (with many previously unreported). Characteristic MS/MS spectra were collected for all fentanyl standards and resulting data indicated that most isomers can be separated via LC (retention times, RT). In other experiments, where analyte separation could not be achieved via RT, analytes were able to be separated in the ion mobility domain. All ionization sources produced analyte species were observed in their protonated form [M+H]1+. A trend was observed where analytes produced a single chromatographic peak but often displayed two ion mobility bands likely do to differing protonation schemes. TIMS spectra showed a typical resolving power of around 100. Inspection of the MS/MS profiles showed similarities across the isomers, highlighting the need for chromatographic or ion mobility separation. Current experiments are focused on the determination of the ionization efficiency, LOD, and reproducibility of the orthogonal ionization sources.

POSTER

P068

Development of SEFRIA Ecstasy Oral Fluid Assay Meeting SAMHSA Guideline

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Abstract

Introduction: 3,4-Methylenedioxymethamphetamine (MDMA), also known as ecstasy, produces euphoria. Methylenedioxyamphetamine (MDA) is a metabolite of MDMA, often sold as ecstasy. Both MDMA and MDA are Schedule I drug under the Controlled Substances Act. Current mandatory guidelines for Federal Workplace Drug Testing Programs include oral fluid testing for MDMA and MDA with initial test cut off at 50 ng/mL and high precision at -/+25% cutoff. Almost identical cross reactivity for both MDMA and MDA is required, which significantly limited choice of antibodies meeting this requirement.

Objective: The objective is to develop a highly sensitive and specific SEFRIA Ecstasy Oral Fluid assay meeting SAMSHA oral fluid drug testing requirement through creative enzyme labeling technology and a specific antibody.

Method: The SEFRIA technology is based on artificial fragments, Enzyme Acceptor (EA) and Enzyme Donor (ED), of the E. coli enzyme β-galactosidase. The assay is based on the competition of the target analyte in an oral fluid sample with the ED-drug conjugate for the fixed amount of antibody binding sites. The target analyte concentration correlates with signal at 570nm. The analytical performance of SEFRIA Ecstasy Oral Fluid assay has been evaluated on the AU5800 chemistry analyzers for precision, specificity and cross-reactivity, interference (non-similar compounds, endogenous substances and commonly ingested substances, pH), linearity, and LCMS method comparison with clinical samples.

Results: The precision study was conducted utilizing 6 controls, 0, 25, 37.5, 50, 62.5, 75ng/ml, with 80 replicates analyzed over 10 days and the assay exhibits <8% CV for semi-quantitative mode and <2% CV for qualitative mode. The assay cross reactivity to MDMA was 142% while using MDA as calibrator. The interference study demonstrated no susceptibility of the assay to interference from 40 structurally unrelated compounds, 10 common endogenous substances, 18 commonly ingested substances at the tested concentration levels, and pH 3.0 to 11. The linear range from 20 to 200 ng/mL was demonstrated by testing recovery of all linearity samples between 85% and 115%, and the %CV < 10%. The method comparison study compared the assay against LC-MS/MS (Liquid Chromatography -Tandem Mass Spectrometry) method. 62 human oral fluid samples containing negative and positive samples were analyzed to show overall agreement at 95.2%, in both semi-quantitative and qualitative analysis to LC-MS/MS.

Discussion: The SEFRIA Ecstasy Oral Fluid assay on the AU5800 analyzers is a reliable assay utilizing oral fluid samples for semi-quantitative and qualitative analysis of MDMA and MDA at 50ng/ml cutoff with controls set at ± 25% of the cutoff.

POSTER

P069

Forensic identification of tobacco products: method development and optimization

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Abstract

Introduction: icotine is one of the most consumed psychotropic substances worldwide. In a large number of jurisdictions, the sale of tobacco and its products is subject to steep taxation. In the province of Québec (Canada), excise and sale taxes make up about half of the final sale price. This makes smuggling operations extremely lucrative and therefore attractive. Such an activity, which can incite increased smoking in the population, can be charged under the Tobacco Tax Act.

Smuggled tobacco is seized in various formats (bulk, cigarettes, chewing tobacco, shisha, etc.) by police officers through a wide array of investigation types. A laboratory-based confirmation of tobacco presence in the products is needed, especially for unusual product types (e.g., shisha/hookah). Unfortunately, the literature on the analysis of tobacco from this specific angle (forensic identification) is scarce.

Objectives: Develop and optimize a cost and time effective accredited method for the forensic identification of tobacco in dried material and shisha.

Methods: A literature review allowed to target the alkaloid compounds nicotine, nornicotine, anabasine, anatabine and cotinine for analysis. Different aspects of a solvent extraction procedure were optimized. Ethanol, acetonitrile, chloroform and dichloromethane:methanol (DCM:MeOH) solvent systems were compared with regards to their extracting power. The use of an initial alkaline digestion step was evaluated, including comparison of different strong bases (NaOH, NH4OH; 0.1N, 3N). Various matrix weight and matrix to extraction solvent ratio were also tested. Finally, both gas chromatography – mass spectrometry (GC-MS) and liquid chromatography – tandem mass spectrometry (LC-MS/MS) were evaluated as instrumental platforms for the detection, and their method parameters were optimized.

Results: The final method is as follows. In a 15 mL polypropylene tube, 1 g of shisha is mixed with 1 mL NH4OH 3 N and vortexed. Sonication is then performed for 20 minutes, and 1 mL of extraction solvent (DCM:MeOH 80:20) is added. After vortexing, centrifugation at 1258xg is carried for 5 minutes. The resulting organic extract, sitting at the bottom of the tube, is collected and diluted by a factor of 5 000 prior to analysis. Nicotine-D4 is used as an external standard (40 ng/mL). A qualitative LC-MS/MS method is used for identification of the targeted analytes, with 5 µL of diluted extract being injected on an Agilent Series 1200/1260 LC coupled to a Sciex 5500 QTrap MS/MS. A 5 minutes chromatography allows baseline separation of all compounds on a Zorbax Eclipse Plus C18 column (2.1 x 100 mm, 3.5 µm), using an aqueous mobile phase of 10 mM ammonium formate pH 9.5 and a methanol organic mobile phase. To meet ASB 113 identification criteria, 3 transitions per analyte are monitored.

Discussion: While some of the selected analytes can be found in other plant materials (e.g., tomato, eggplant) at low levels, taken together, the presence of all five alkaloids is characteristic of tobacco. While GC-MS analysis was found to be possible with the undiluted organic extract, the high load in matrix components created robustness issues. The increased sensitivity brought by LC-MS/MS allowed further dilution, thus solving all matrix effect issues. The final method is cost effective, using a low amount of solvent and very little consumables – elements which are also environmentally friendly. A potential change in the solvent extraction system could make the method even greener and facilitate manipulations, if the selected solvent had a density lower than water. The rapid extraction combined with the short chromatography also makes this a time effective procedure. This is compounded by an automated generation of the expertise reports based on the instrumental output. This method will be included in the December 2023 accreditation scope.

POSTER

P070

Validation of a Simple Chromatographic Method to Screen Oral Fluid Samples for Tier I Drugs in DUID Investigations

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Abstract

Introduction: Oral fluid has become a desirable drug testing matrix for impaired driving (DUID) investigations. A simple and efficient sample collection process, along with interpretive advantages over urine, further promote its utility for this application. Since oral fluid sample collections yield low volumes (typically ≤ 4 mL) and contain low drug concentrations, additional analytical factors must be considered when establishing reliable testing methods. Given these considerations, chromatographic screening methods have become increasingly prevalent alternatives to immunoassay techniques. The sensitivity and selectivity of targeted methods allows for lower detection limits and increased flexibility in the testing scope. The National Safety Council's Alcohol, Drugs, and Impairment Division (NSC-ADID) has established scope and sensitivity recommendations for oral fluid testing as a starting point for laboratories introducing this matrix. Since this method is a targeted chromatographic screen, thresholds were set to match the confirmation levels in the guideline.

Objectives: To develop and validate a simple liquid-liquid extraction (LLE) and chromatographic method for oral fluid screening which is compliant with published Tier I DUID scope and sensitivity recommendations (2021 update). To provide as much differentiation as possible from subsequent confirmation methods by varying extraction technique, target MRM transitions, and chromatographic column.

Methods: Sample preparation involved a single-step LLE applied to samples collected with the QuantisalTM device. Oral fluid samples (400 µL) were adjusted to a basic pH with 250 µL of 10% ammonium hydroxide and combined with 1.5 mL of 50:30:20 methyl tert-butyl ether:isopropanol:hexane. After mixing and centrifugation, the organic layer was removed and 50 µL of 1% hydrochloric acid in methanol was added to stabilize amines. Extracts were then evaporated to dryness and reconstituted with 50 µL of 95:5 mobile phase A1:B1. Instrument analysis was conducted using a Waters Acquity I-Class liquid-chromatograph coupled to a Xevo TQ-XS tandem mass spectrometer (LC-MS/MS). A Restek RaptorTM biphenyl column (2.1 mm x 50 mm, 1.8 µm particle size) was used for chromatographic separations, with an injection volume of 3 µL. Mobile phases consisted of 0.1% formic acid in water (A1) and 0.1% formic acid in 50:50 acetonitrile: methanol (B1), and the total run time was 8 minutes. Qualitative validation experiments were performed according to ANSI/ASB Standard 036 – Standard Practices for Method Validation in Forensic Toxicology.

Results: Interference study results demonstrated good method selectivity. Detection of cannabis was targeted to tetrahydrocannabinols; isomer separation is accomplished in the confirmation method. Ion suppression/enhancement (ISE) values for all analytes were similar to their corresponding internal standards (IS), indicating that ISs sufficiently mitigated any matrix effects. Some ISE values exceeded the ±25% threshold, including THC which showed a significant ion enhancement effect. Limit of detection studies included nine internally collected authentic human matrix sources, and results demonstrated sufficient signal-to-noise ratios, precision, and reproducibility at the Tier I confirmation thresholds. Extracts were stable over 48 hours, and analyte carryover was mitigated using solvent blanks. Additionally, the method was successfully applied to reference laboratory and proficiency samples. All screening results agreed with sample provider results, except for two clonazepam positives and one oxymorphone positive which screened below the cutoffs for this method.

Discussion: A rapid, robust, and efficient chromatographic method with a unique LLE approach was validated to screen oral fluid samples for the Tier I compounds (and phencyclidine) at the confirmation cutoffs recommended by the NSC-ADID for DUID testing. The method allows for all analytes to be screened in a single test using minimal sample volume (400 µL). The use of LLE is simple, rapid, and allows for further differentiation from the confirmation methods which utilize solid phase extraction.

POSTER

P071

Human Factors May Influence THC Capture with an Impaction Filter Device: Towards Standardized Sampling and Calibration Protocols

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Abstract

Introduction: Breath-based measurements of recent cannabis use could be a valuable tool to deter and determine cannabis-impaired driving. THC (delta-9-tetrahydrocannabinol), the primary psychoactive molecule in cannabis, is a large molecular weight compound that is present in trace concentrations in breath. Based on its low volatility [1], THC is hypothesized to be carried by aerosol particles formed within the lungs and pilot-scale studies have quantified THC captured by filter-based devices. There are currently no standardized sampling protocols or calibration materials with which to validate these devices and build public acceptance.

Objectives: We recently quantified THC in breath samples collected with an impaction filter device before and after participants smoked legal-market cannabis (25% THC-A) within a naturalistic study of cannabis and anxiety [2]. Breath was sampled 1 h to 1.5 h after cannabis use, when individuals may begin to feel comfortable driving [3]. We developed computational fluid particle dynamics models to examine whether the device's mode of action is dependent on flowrate, which may vary from sample to sample.

Methods: While the complete device comprises three impaction filters, we simulated aerosol capture within one filter. We considered two phases: a fluid phase consisting of air saturated with water vapor (6%) and a discrete phase consisting of aerosol particles with the physical properties of water droplets (5000/L). The simulated particles had diameters of 0.48 µm (51.9% of the particles), 0.63 µm (24.7%), 0.81 µm (15.6%), and 1.18 µm (7.8%). Boundary wall conditions were set so that a particle was "captured" upon contact with a filter surface. If a particle reached the filter outlet without contacting any filter surface, then it was considered "lost" from the filter. Particles could be deposited via impaction, interception, and/or turbulent dispersion due to continuous fluctuating fluid forces or eddies. We simulated five flow rates through the filter: 0.02 L/s, 0.2 L/s, 0.4 L/s, 0.8 L/s, and 1.2 L/s.

Results: THC quantities recovered from the impaction filter device 1 h to 1.5 h after cannabis use were broadly comparable to previous pilot studies, which include order-of-magnitude differences between studies and within studies [2]. Numerical simulations demonstrate the dramatic effect of flowrate on the efficiency of particle capture. At 0.02 L/s, low velocities hinder the deposition of even the largest particles and particles are relatively uniformly distributed throughout the filter. Compared to the other four flowrates, a significant fraction of particles is lost. At this flowrate, the primary deposition mechanism is, in fact, interception. By comparison, at 0.4 L/s, high velocities promote the deposition of particles within the first third of the filter, particularly for the larger particles, and the primary deposition mechanism is impaction.

Discussion: Our results suggest that aerosol capture by impaction filter devices is strongly influenced by flowrate, therefore order-of-magnitude differences in THC quantities in authentic samples may be partially attributable to differences in breath sampling. We will discuss the potential for spirometry to offer insight into the consistency of samples collected at different timepoints or with different participants and an upcoming study that investigates the feasibility of a two-point measurement to identify recent use.

References:

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POSTER

P072 - Withdrawn

P073

Reference concentrations and distribution of 7-aminoclonazepam in hair following a single dose of clonazepam

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Abstract

Introduction: The benzodiazepine clonazepam is frequently implicated in cases of drug facilitated crimes (DFC). In DFC cases where the substance is no longer detectable in blood and urine, hair analysis is an important tool to reveal an exposure to clonazepam. To distinguish single from repeated doses, established reference concentrations of clonazepam and the metabolite 7-aminoclonazepam in hair following a controlled single-dose are essential.

Objectives: The objective of this study was to establish reference values of clonazepam and 7-aminoclonazepam in hair following a single dose of clonazepam and to investigate the time-resolved drug distribution across hair samples collected at four different sampling times.

Methods: Eighteen healthy volunteers (15 females and 3 males) received a single dose of either 0.5 (n=8) or 2 mg clonazepam (n=10). Hair samples were collected from each participant 14, 30, 60 and 120 days after administration from the posterior vertex as close to the scalp as possible.

The study protocol was approved by the Reginal Ethics Committee in Linköping, Sweden (number 2010/41-31) and all participants gave their informed consent to participate in the study.

The hair extracts were pulverized and incubated in extraction medium (methanol, acetonitrile, 2 mM ammonium formate, 25:29:46, pH 5.3) overnight at 37°C. After filtration, the hair extracts were analysed by ultra-high performance liquid chromatography–tandem mass spectrometry using a validated limit of quantification of 1.0 and 0.5 pg/ng for clonazepam and 7-aminoclonazepam, respectively.

Results: ll analyzed hair samples were positive for 7-aminoclonazepam, while clonazepam could not be detected in any of the hair samples. Assuming an average hair growth rate of 1 cm/month, the highest concentration of 7-aminoclonazepam was found in the expected segment in most of the hair samples with lower concentrations in the adjacent segments.

The time-resolved drug distribution profiles across the four sampling time points showed that the highest concentrations were detected in the hair samples collected after 30 and 60 days. The lower concentrations in hair samples collected after 14 days can be explained by the fact that there is still some positive hair below the surface of the scalp and that hair was cut 1-2 mm above the scalp. In hair samples collected after 120 days, the drug was more dispersed in two segments due to irregular hair growth.

In the hair samples collected after 30 and 60 days, the concentrations of 7-aminoclonazepam ranged from 1.1 to 10 pg/ng (median: 2.1 pg/ng) in the low-dosing group (0.5 mg), while the concentrations in the high-dosing group (2 mg) were 3-4 times higher ranging from 2.2 to 35 pg/ng (median: 9.6 pg/ng).

The cumulative concentrations for each sampling time were lowest at the first sampling time and consistent across the three later sampling times, indicating that 7-aminoclonazepam is not subject to significant sweat contamination or washout over time.

Conclusion: Reference values were established for 7-aminoclonazepam in hair from 18 participants who ingested a single dose of either 0.5 or 2 mg clonazepam, while clonazepam was not detected. This shows that it is essential to include this metabolite in the analysis to confirm an exposure of clonazepam.

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The time-resolved drug distribution profiles across the four different sampling time points showed that analysis of 1 cm segments can assist to determine the time of a single exposure of clonazepam and that the hair sample should be collected 1-2 months after the exposure to provide the best conditions for detection and interpretation of the hair results.

P074

Immobilized Enzymes on Magnetic Beads for Separate Mass Spectrometric Investigation of Human Phase II Metabolite Classes

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Abstract

Introduction: The clearance of xenobiotics in the human body is a multistep process consisting of phase I and phase II modifications. These biotransformations require a series of enzymes that convert the non-endogenous metabolites into compounds with higher hydrophilicity. Xenobiotics are important for diverse research fields such as biomarker discovery, toxicology, nutrition, doping control, and microbiome metabolism. The two major phase II modifications, sulfation and glucuronidation, have been linked to microbiota-human host co-metabolism. Furthermore, the importance of the microbiome metabolism on the conversion of dietary compounds has been revealed to impact human physiology including the production of potential toxins.

Objectives: One of the major challenges in the field has been the development of methodologies for advanced targeted investigation of glucuronidated and sulfated metabolites. We aim to develop a chemical biology tool for the separate investigation of these two phase II modifications and their corresponding unconjugated aglycons utilizing recombinant enzymes in collaboration with Kura Biotech.

Methods: Our new methodology utilizes an immobilized arylsulfatase (ASPC™) and an immobilized β-glucuronidase (BGTurbo®, B-One®) to magnetic beads for treatment of human urine samples obtained from a dietary intervention study. After the selective conversion of sulfated and glucuronidated metabolites using these immobilized recombinant enzymes, the samples were subjected to UHPLC-MS/MS analysis. The obtained raw data were then processed with R using the XCMS metabolomics framework to selectively identify metabolites with a sulfate and glucuronide moiety. The metabolite structure was either validated via authentic standards or MS/MS fragmentation analysis.

Results: The separate mass spectrometric investigation of each metabolite class in a single sample was successfully applied to obtain the dietary glucuronidation and sulfation profile of about 100 compounds. We were able to relatively compare the quantities of each metabolite conjugate as well as the aglycon within the sample and observe the changes in the metabolic profile before and after the dietary intervention.

Discussion: This methodology allows for the identification of the metabolite structure for all three metabolite forms (unconjugated, glucuronidated and sulfated) in the same urine sample. Our new chemical biology strategy provides a new tool for the investigation of metabolites in biological samples with the potential for broad-scale application in toxicology, metabolomics, nutrition and microbiome studies.

POSTER

P075

Identification of “Nitazenes” in whole blood samples by LC-MS/MS and LC-HRMS/MS

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Abstract

Introduction: The non-fentanyl derived synthetic opioids family represents a wide variety of synthetic opioids including the 2-benzylbenzimidazole analogs also known as “nitazenes”. Due to the rapid emergence of “nitazenes” in the drug market and the increase of opioid related deaths over the years, it was crucial to have these drugs included in our routine screening method.

Objectives: A validation following the standard practices delineated on ANSI/ASB STANDARD 036 was performed using blood specimens to identify five analogs: etodesnitazene, protonitazene, N-pyrrolidino etonitazene, metonitazene, and etonitazene.

Methods: The drugs were extracted from the biological matrix by protein precipitation. Aliquots of 1 mL were used for liquid chromatography tandem mass spectrometry (LC-MS/MS) analysis using ABSciex 3200 QTRAP mass spectrometer equipped with an Agilent Eclipse Plus C18 2.1 x 50mm 3.5 micron column. Additionally, aliquots of 0.5 mL were extracted and analyzed on a liquid chromatography Thermo Scientific Q Exactive Focus high resolution accurate mass spectrometer (LC-HRMS/MS). Chromatographic separation was performed using an Agilent Poroshell 120 EC-C18 2.1x100 mm 2.7 micron column fitted with a matrix matching guard column. To ensure confidence and reliability of the validation different parameters including interference, ionization/suppression enhancement, limit of detection (LOD) and carryover were evaluated.

Results: Potential causes of interference were evaluated including related compounds and isotopes. All analytes produced fully resolved peaks at different retention times (RT) enough to differentiate between compounds. LOD were established at 3.0 µg/L on LC-MS/MS and 2.0 µg/L on LC-HRMS/MS for all but one analog. Protonitazene LOD was 3.0 µg/L on LC-MS/MS and 5.0 µg/L on LC-HRMS/MS. Fortified samples at concentration of 0.50 mg/L (LC-MS/MS) and 0.10 mg/L (LC-HRMS/MS) were analyzed in triplicate with subsequent blank matrix samples injected to determine risk of carryover. No carryover effects were detected in any of the blank samples.

Discussion: The laboratory’s routine screening methods proved suitable to detect and identify all five 2-benzylbenzimidazole analogs. As a result of successful validation, the methods were implemented in the analysis of authentic case samples.

POSTER

P076

In Vitro Formation of Hydroxy Metabolites in Hair after Hair Product Exposure

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Abstract

Introduction: Hydroxy metabolites of cocaine and amphetamines have been suggested as possible targets for hair testing that may differentiate between drug use and external contamination. There is uncertainty regarding the ability of common hair treatments, such as bleaching and relaxing, to convert parent drug compounds into hydroxy forms in vitro. If hydroxy metabolites can be formed in vitro from common hair treatments on externally contaminated hair, they may not be suitable to demonstrate actual drug use or may have only limited use. One such suggested use is utilizing ratios of these metabolites relative to cocaine versus the absolute concentration of these metabolites.

Objective: The objective of this study is to further our understanding of possible in vitro formation of hydroxy metabolites of cocaine, amphetamine, and methamphetamine when the respective parent compounds are exposed to the oxidizing conditions of common hair treatments.

Methods: Drug-free human hair was collected from volunteers and potential drug positive human hair was obtained from a local substance abuse treatment center, both under IRB-approved protocols. Drug-free hair was separated into three batches. One batch was externally contaminated with cocaine and amphetamine, one was contaminated with benzoylecgonine (BZE) and methamphetamine, and the third batch served as a negative control and was not contaminated. Hair was contaminated by incorporating 5mg of each drug in powdered form into 4g of hair, applying a coating of synthetic sweat, letting the hair sit at room temperature for 48 hours, then rinsing off excess drug with water. Samples from each batch were extracted and analyzed for relevant compounds using LC-MS/MS before and after exposure to three hair care products: a no lye relaxer, an extra light blond hair dye, and a medium golden grown hair dye. Hair was extracted using methanol with 1% HCl prior to solid phase extraction.

Results: Ortho, meta, and para-hydroxy cocaine, cocaethylene, and norcocaine were identified in hair contaminated with cocaine both before and after exposure to the hair care products. Preliminary data indicates that before exposure to the hair care products average metabolite to cocaine ratios were 0.014%, 0.013%, 0.011%, 0.668%, 1.115% for ortho, meta, and para-hydroxy cocaine, cocaethylene, and norcocaine. After exposure the average ratios were 0.018%, 0.023%, 0.019%, 0.740%, 2.344%.Para-hydroxy BZE was identified in hair contaminated with BZE before and after exposure to hair care products with preliminary average para-hydroxy BZE to BZE ratios of 0.012% and 0.018%, respectively. A small amount of 4-hydroxy methamphetamine was identified in hair contaminated with methamphetamine both before and after exposure to the hair care products with metabolite to methamphetamine ratios of 0.003% and 0.007% (average), respectively.

Discussion: Trace amounts of hydroxy metabolites appear to be formed in contaminated hair with and without exposure to hair products. The lowest ratio that has been proposed for identification of drug use versus contamination is 0.05% for para- and meta-hydroxy cocaine. Preliminary results for % para- and % meta-hydroxy cocaine versus cocaine did not reach a concentration that would be consistent with drug use. Ortho-hydroxy cocaine was also below this proposed ratio. Cocaethylene and norcocaine ratios were higher, but there is no proposed use ratio established for these metabolites yet.

POSTER

P077

Long-term Volatile Stability in Whole Blood Containing Different Additives and Assessment for Isobutylene Interference in BD Vacutainer® Tubes

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Abstract

Introduction: While gray top tubes are preferred for analysis of volatiles in blood, the evaluation of other blood collection tubes may provide a suitable alternative if gray top tubes are unavailable. In 2022, Becton Dickinson (BD) released an advisory to stakeholders that their 10mL gray top Vacutainer® tubes contain isobutylene, which may potentially interfere with methanol analysis by gas chromatography with flame ionization detection (GC-FID).

Objectives: The purpose of this study is to provide an update on the long-term stability of common volatiles in whole blood when stored in five different color top tubes and assess the samples for isobutylene interference.

Methods: Gray (10mL), lavender (4.0mL), pink (2.0mL), light blue (2.7mL), and clear (6.0mL) top BD Vacutainer® blood collection tubes were filled to approximately 75% (50% for pink) of their fill volume with whole blood containing citrate phosphate dextrose additive fortified with 0.08g/100mL ethanol, methanol, isopropanol, and acetone using a syringe. Samples were stored under refrigerated and room temperature conditions and analyzed in triplicate using dual-column headspace GC-FID at 9 timepoints over 1 year, with a LOQ of 0.01g/100mL for all analytes. Acceptable stability is defined by the uncertainty of measurement for each analyte (9.6, 11, 9.7, and 12%, respectively as previously listed).

Isobutylene samples were prepared by fortifying negative controls with isobutylene standard (2-methylpropene, 99%) at 0.00371% and 0.00668% v/v. Each concentration was analyzed in triplicate to determine the retention time (RT). Samples containing 0.0192g/100mL ethanol, methanol, isopropanol, and acetone (LMQC) were fortified with isobutylene to assess for significant interference with quantitation (>10% difference from target).

Results: Gray tops showed acceptable stability for all analytes up to 1 year under both storage conditions. Under refrigeration, analyte stability ranked (ethanol, methanol, isopropanol, acetone): lavender (6months, 1year, 1year, 1year) > pink (6months, 1year, 1year, 4months) > clear (6months, 1year, 1year, 4months) > light blue (1day, 6months, 1day, 1day). The same general trend was observed at room temperature, but with pink showing a greater decrease in stability relative to lavender. Light blue showed unacceptable stability at 1 day for all analytes except methanol.

The RT of isobutylene was determined to be 0.964 min (Rtx-BAC Plus 1) and 0.743 min (Rtx-BAC Plus 2). LMQC samples fortified with isobutylene showed no significant interference with quantitation of the analytes of interest. Isobutylene was not identified in any of the samples stored in the BD Vacutainer® tubes analyzed throughout the volatile stability study.

Discussion: Relative to gray tops, lavender and pink tops showed the most comparable stability for all analytes under refrigeration. However, under room temperature conditions, lavender tops showed greater stability than pink tops for ethanol, isopropanol, and acetone, despite having the same type and proportion of additive. This finding supports refrigerated storage as a key component in maintaining analyte stability. The immediate decrease in analyte stability in light blue tops may be attributed to the liquid additive diluting the samples.

The RT of isobutylene was outside of the RT window (±1%) for all analytes of interest. As such, the HFSC Volatiles method does not misidentify isobutylene as methanol, a potential risk presented in the BD advisory notice. However, insufficient resolution between methanol and isobutylene may be observed on Rtx-BAC Plus 1. If unacceptable resolution between isobutylene and methanol were to be observed in repeated analysis of a sample, said sample should be considered unsuitable for quantitation of methanol per laboratory policy. The absence of isobutylene in the samples stored in BD Vacutainer® tubes used for the stability study may potentially be attributed to lower instrument sensitivity for isobutylene.

POSTER

P078

Prevalence of opioid use among gabapentin positive urine specimens

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Abstract

Introduction: Gabapentin was approved by the US Food and Drug Administration (FDA) for the treatment of partial seizures in 1993, and subsequently for postherpetic neuralgia in 2002. Because gabapentin was considered safe and was assumed to have low potential for abuse, the drug was prescribed off-label for conditions such as diabetic neuropathy, restless legs syndrome, and migraine headache. In recent years, misuse of gabapentin with associated harm has been reported, especially when co-ingested with central nervous system (CNS) depressants such as opioids. In December 2019 the FDA required new warnings about the risk of respiratory depression in patients who co-ingest gabapentin with CNS depressants, have underlying respiratory impairment, and the elderly.

Objectives: The objective of this study was to determine the prevalence of opioids (6-acetylmorphine [6-AM], morphine, codeine, hydrocodone, hydromorphone, and norhydrocodone) in urine from patients who tested positive for gabapentin during routine urine drug testing as a component of a behavioral health, pain management, or addiction treatment program.

Methods: Data from consecutive random urine specimens submitted for drug monitoring from January 1st to March 31st, 2023 were deidentified and positivity for gabapentin and opioids assessed.

Specimens had been tested using CLIA-validated laboratory-developed, liquid chromatography tandem mass spectrometry (LC-MS/MS) assays. Morphine, codeine, hydrocodone, hydromorphone, and norhydrocodone were run in a panel (opiate panel), whereas gabapentin and 6-AM were assayed using separate methods. Cut-off concentrations for positivity were 50 ng/mL for all opiate panel analytes, 1,000 ng/mL for gabapentin, and 10 ng/mL for 6-AM. The upper limits of the analytical measurement ranges (AMR) were 10,000 ng/mL for opiate panel compounds, 200,000 ng/mL for gabapentin, and 2,000 ng/mL for 6-AM.

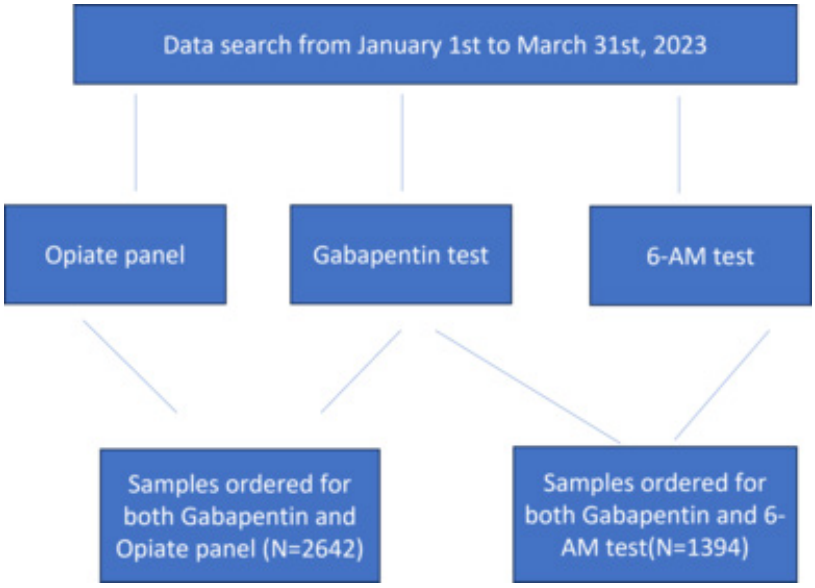
Results: During the study period 2,642 specimens were assayed using both gabapentin and the opiate panel. Of the specimens tested for gabapentin, 799 (30.2%) were positive and 49 (6.1%) of these positive specimens were also positive for ≥1 opiate, including 21 (2.6%) for morphine with mean concentration of 1450 ng/mL, 17 (2.1%) for codeine with mean concentration of 704 ng/mL, 19 (2.4%) for hydrocodone with mean concentration of 957 ng/mL, 24 (3.0%) for hydromorphone with mean concentration of 454 ng/mL, and 21 (2.6%) for norhydrocodone with mean concentration of 1257 ng/mL. All opiate results were within the AMR except for seven specimens with concentrations >AMR for both gabapentin and morphine.

In the same study period 1,394 specimens were assayed for both gabapentin and 6-AM. Of the specimens tested for gabapentin, 378 (27.1 %) were positive and 2 (0.5%) of these were also positive for 6-AM at a concentration of 50 and 201 ng/mL.

Conclusion/Discussion: Gabapentin was detected in about 30% of the specimens submitted for drug monitoring and concomitant use of opiates was detected in about 6% of these specimens. Hydromorphone was the most prevalent (3% of gabapentin-positive specimens) and 6-AM the least prevalent (0.5%). Moreover, 7 specimens exhibited high concentrations of both gabapentin and morphine, possibly reflecting misuse. These data suggest that among gabapentin positive samples in the study population, the misuse of heroin is low. While this study contributes valuable insights into the prevalence of gabapentin use and its association with select opioids, its findings are limited by the study population and absence of other opioids such as oxycodone and fentanyl from the panel.

POSTER

Figure 1 Study design



POSTER

P079

Evaluation of a fast and novel workflow for urine toxicology screening with DART-MSMS and Toxbox®

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Abstract

Introduction: Conventional forensics toxicology urine screen often employ immunoassay which are prone to false negatives, cross talk and a variety of other issues. Further, additions of new test targets are slow and cannot adequately respond to the fast-moving introduction of new substances in today's world. Herein, we report on a rapid, simple, mass spectrometry based urine assay vastly improving screening capabilities and amenable to emerging drug targets.

Objectives: Combining Direct Analysis in Real Time-MSMS (DART-MSMS) with ToxBox® (PinPoint Testing, LLC) offers a fast and simple solution for a wide audience urine screening workflow. The primary goal is to provide enhanced data quality by relying on mass spectrometry, with analysis time similar to immunoassay. The secondary goal is to simplify the urine screening workflow by including high quality commercial kits to ease a transition to mass spectrometry from immunoassay

Methods: Screening workflow includes the following groups, 1 (amphetamine, methamphetamine, MDA, MDEA), 2 (codeine, hydrocodone, oxycodone, oxymorphone, morphine, hydromorphone, tapentadol, methadone, tramadol), 3 (diazepam, flurazepam, clonazepam, alprazolam, lorazepam, temazepam, triazolam, oxazepam), 4 (7-aminoclonazepam, alpha-hydroxymidazolam, 2-hydroethylflurazepam, 7-aminoflunitrazepam, desalkylflurazepam, alpha-hydroxyalprazolam), 5 (benzoylecgonine, PCP, mitragynine, 6-MAM, THC-COOH), 6 (fentanyl, acetylfentanyl, 3-methylfentanyl, nor-fentanyl).

Development: DART parameters (temperature, scan type, scan time) were optimized to maximize both sensitivity and precision. All drugs were tuned. Calibration series, QCs were prepared in pooled human urine, with selected deuterated IS, by PinPoint Testing, LLC (ToxBox®). Liquid-liquid extraction was performed manually, concentrating the organic layer (2h preparation time). This step can be automated. 1 µL aliquots were loaded onto a 96-mesh plate and transferred to DART-MS for analysis (20-30 seconds per sample, total run time < 1hr.

Validation: Regression curves were run, each comprised of 8 calibrators, blanks, matrix blanks and 4 QC in quadruplicate. Each set was run multiple times. LOD/threshold, carry-over, and interference studies were completed.

Results: Data were processed via standard MS quantitation software, using internal standards. Drugs were analyzed by groups for DART-MSMS: (1) amphetamines, (2) opiates, (3) benzodiazepines, (4) benzodiazepines metabolites, (5) illegals, and (6) fentanyls. Run time for each group was 20-30 seconds per sample and samples were in 96 sample plate configuration. Total plate run time was under 1h. DART parameters were optimized for each group. Helium stream temperature was determined to be as follow: (1) 200 C; (2) 350 C; (3) 450 C; (4) 450 C; (5) 450 C; (6) 450 C. All groups were run in pulse mode, 5-8 seconds duration.

All drugs investigated for this semi-quantitave assay were analyzed via selected quadratic regression curves in the range tested, with R2 values of 0.985 or better for screening. Quadratic regression was selected to extend the screen range. Precision and bias were reported for all QC's. Most drugs passed the validation protocol, following traditional FDA requirements.

Discussion: This method describes an approach for screening urine toxicology samples by mass spectrometry. Decreased turnaround time, simplicity, data quality matches and exceed current criteria for screening assays. Furthermore, this solvent free and chromatography free approach reduces environmental impact. The use of ToxBox® furthers simplifies the workflow and renders mass spectrometry more accessible. New target analytes can be added to the assay by simply adding the appropriate MS transitions, selected internal standard transitions, and verifying that the new analyte abides by the validation criteria. Typically, a new validation exercise is performed.

Analysis of a correlation set against a validated assay is pending.

POSTER

P080

Rapid and Sensitive Quantification of Psilocin in Human Whole Blood using Liquid Chromatography Tandem Mass Spectrometry (LC-MS/MS)

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Abstract

Introduction: Psilocybin is the principal component found in *Psilocybe* mushrooms that undergoes first-pass liver metabolism to form the pharmacologically active Psilocin. Recent clinical advancements have demonstrated the potential of psilocybin in treating major depressive disorder, antidepressant-resistant depression, and various neurological and neurodegenerative conditions. Consequently, there has been enthusiastic legislative progress for psilocybin-facilitated therapies, with Oregon endorsing controlled psilocybin administration as psychotherapy adjuncts, and similar reforms being introduced throughout the U.S. However, psilocybin's rising popularity may lead to a corresponding increase in illicit mushroom abuse as psilocybin and psilocin are still Schedule I drugs under the Controlled Substances Act. This is of forensic concern because psilocybin's pharmacodynamic/pharmacokinetic (PD/PK) profile and long-term side effects remain poorly understood. Thus, it is critical to develop a useful analytical workflow for both clinical and forensic toxicological testing. The aim of this study is to develop and validate an extraction and analytical assay that can detect and quantify psilocin in human whole blood.

Objectives: The goals are: (1) to optimize a whole blood extraction with high psilocin recovery but low matrix effects, and (2) to develop a sensitive LC-MS/MS method to quantify psilocin.

Methods: Psilocin was isolated from 0.5 mL of human whole blood using solid-phase extraction (SPE) and psilocin-d_{10} as the internal standard (ISTD). This extraction included three simple washes before analytes were desorbed off the Cerex® Clin II SPE columns with 2% ammonium hydroxide in ethyl acetate. All analysis was performed using an Agilent 1290 Infinity II Liquid Chromatograph coupled to an Agilent 6470 Triple Quadrupole Mass Spectrometer. The LC method had a run time of 6 minutes using gradient elution. An Agilent Infinity Lab Poroshell 120 EC-C18 with matching guard column, and mobile phases (A) 5 mM Ammonium formate with 0.01% formic acid in diH_2O, and (B) 0.1% formic acid in ACN at a flow rate of 0.5 mL/min, was utilized for psilocin elution. Electrospray ionization was in positive mode, and the mass analyzer was operated in the multiple reaction monitoring mode with one quantifier and qualifier transition for psilocin and ISTD identification. The heavier m/z fragments were quantifiers due to their reduced susceptibility to matrix effects while maintaining good chromatography and high signal. Full quantitative method validation was performed according to ANSI/ASB 036 guidelines.

Results: All validation parameters met ANSI/ASB 036 acceptability criteria. SPE recovery efficiency was 89.9-95.2%. Linearity was established between 0.78-200 ng/mL using a curve weighting factor of 1/x, and the LOD/LOQ determined to be 0.78 ng/mL with negligible carryover. Across all QCs (2, 20, 160 ng/mL), bias ranged from -9.6% to 16.6%, while within-day and between-run precision was within 2.5-15.5% and 9.1-11.5% respectively. 2-fold dilution (80 ng/mL) integrity studies yielded a bias between -2.9-16.9%, within-day %CV of 3.5-17.9%, and between-run %CV at 12.2%. Matrix effects characterized using post-extraction addition at the low and high QCs were found to be 15.1% and -3.1% respectively and were sufficiently accounted for with the deuterated ISTD. Positive controls fortified with 176 common drugs of abuse, and negative controls were free of exogenous/endogenous interferences. Finally, blood extracts were still stable beyond 48 hours when stored in the autosampler (4°C).

Discussion: With whole blood routinely encountered in forensic toxicology casework and directly collected in clinical studies, this workflow enables a fast, two-pronged application that removes any pretreatment needed to isolate plasma/serum. Additionally, the measurement of total psilocin content in blood directly correlates to physiological changes in controlled psilocybin administrations, yielding supplementary results to that of plasma-derived data and ultimately providing a more comprehensive PD/PK profile of psilocybin.

POSTER

P081

It's Not Just Fentanyl Anymore: Implications for Drug Delivery Resulting in Death Cases

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Abstract

Introduction: Drug-induced homicide holds individuals responsible for supplying drugs that result in a fatal overdose even in the absence of the intent to kill. Within the United States laws vary state-by-state, but in general require prosecutors to establish that the drug (most commonly fentanyl) delivered by the individual being prosecuted was responsible for death.

Objectives: This study examines a large data set of postmortem toxicology cases to determine the frequency of concomitant drug use in individuals who were exposed to fentanyl at or around the time of death to highlight the difficulty in interpretation related to drug-induced homicide cases.

Methods: Results for any blood fentanyl-positive postmortem cases received from 2018 through 2022 were evaluated for the presence of additional findings after comprehensive toxicology testing performed at a large reference laboratory. Data was evaluated for frequency of each individual drug associated with fentanyl-positive cases as well as the frequency of combinations of drugs. In addition to other drugs of abuse, prescribed drugs were also considered.

Results: Over the 5-year study period 343,632 cases were analyzes; 105,121 (30.6%) were positive for fentanyl. The percent positive increased each year from 21.3% in 2018 up to 35.2% in 2022. Only 31,064 cases were positive for fentanyl only.

Table 1 lists the most common findings for the 74,057 cases that were positive for at least one drug in addition to fentanyl.

Table 1

Analyte	N	%
Cocaine / BZE	31553	42.6
Cannabinoids	29263	39.5
Methamphetamine	27097	36.6
Ethanol	25187	34.0
Naloxone	23468	31.7
Heroin*	13828	18.7
Alprazolam	10820	14.6
Diphenhydramine	10723	14.5
Gabapentin	9341	12.6
7-Amino Clonazepam	8069	10.9
Morphine	7943	10.7
Xylazine	7388	10.0

*Heroin = Morphine positive blood + 6-acetylmorphine in any specimen

The most common combinations of drugs (excluding cannabinoids and ethanol) are indicated in Table 2.

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Table 2

Fentanyl Alone and in Combination	N	%
Fentanyl Only	31064	29.6
Fentanyl, Cocaine	17958	17.1
Fentanyl, Methamphetamine	15548	14.8
Fentanyl, BDPs	12274	11.7
Fentanyl, Heroin	5025	4.8
Fentanyl, Cocaine, BDPs	4568	4.3
Fentanyl, Methamphetamine, BDPs	4311	4.1
Fentanyl, Cocaine, Methamphetamine	3337	3.2
Fentanyl, Cocaine, Heroin	2830	2.7
Fentanyl, BDPs, Heroin	2124	2.0
Fentanyl, Methamphetamine, Heroin	1543	1.5

Table 3 includes drug classes detected more than 20% of the time with fentanyl. Note that more than one drug in each class may be present.

Table 3

Class	N	%
Stimulant*	62416	84.9
Opioid / NPS Opioid	46034	62.6
BDP / NPS BDP	32769	44.6
Cannabinoids	29263	39.8
Various Prescribed Medications**	25318	34.4
Ethanol	25187	34.3
Narcotic Antagonist	23789	32.4
Antidepressant / Psychotic Non-SSRI	20227	27.5
Serotonin Reuptake Inhibitors (SSRI)	17447	23.7

*Stimulant = Methamphetamine, Amphetamine (without Methamphetamine), MDMA, and Ritalinic Acid

**Prescribed medications other than antidepressants and antipsychotics

Discussion: In a large study of fentanyl-related postmortem cases, fentanyl was frequently detected with other drugs. This can complicate drug delivery resulting in death cases especially when the uningested delivered drug(s) have not been tested. Frequently, it is not known whether detected drugs were consumed at or near the same time or the role the different drugs present may have had in contribution to death.

When considering drug classes detected more than 20% of the time with fentanyl, other drugs of abuse were the most frequently detected. However, a significant number of cases included various prescription medications, and antidepressants and antipsychotics (of both the SSRI and non-SSRI type). The latter agents may contribute by enhancing sedation, hypotension, respiratory depression, and QT prolongation. These results emphasize the importance of considering the role concomitant drug use may play in a death when there are charges of drug delivery in conjunction with, hopefully, the context of autopsy findings.

P082

What is the Preferred Educational Background of Forensic Scientists?

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Abstract

Introduction: Forensic scientists straddle the realms of basic and applied science, applying fundamental principles of biology, chemistry, mathematics, and physics while aiding in investigations of crimes. Most practitioners receive an undergraduate education in a science, technology, engineering, or mathematics (STEM) discipline and learn how to apply this knowledge on-the-job through lengthy apprenticeship-style training programs. Studies published in 1988 and 1996 quantified the preferred or required educational backgrounds of forensic scientists. However, since its establishment in 2002, the Forensic Education Programs Accreditation Commission (FEPAC) has accredited educational BS and MS programs and contributed to an overall increase in the quality of available forensic science education. Increasing the availability of high-quality forensic science educational programs is beneficial to both the forensic science community and the public; however, since FEPAC's inception, few studies have re-surveyed crime lab directors regarding their preferences for candidate educational degrees.

Objectives: In this work, we sought to re-examine the preferred degrees held for both entry-level and supervisory forensic science candidates. Survey questions were designed to identify the impacts of FEPAC accreditation has had on the desirability of certain educational degrees.

Methods: Data were gathered through an electronic survey distributed to crime laboratory directors, members of leadership, and hiring managers. The survey was distributed via anonymous link, and responses were only accessible to those with survey administrator log-in credentials. Survey questions paralleled those used by Siegel's 1988 publication and asked what educational background respondents prefer in entry- and supervisory-level candidates and how often the selected candidate has that educational background. Respondents were asked to rank from 1 (most preferred) to 7 (least preferred) various hypothetical educational backgrounds of entry-level and supervisory-level candidates. After each question about preferred candidate background, respondents were asked how often hired candidates had their preferred educational background. Respondents were given the following options: all the time (90–100% of new hires), often (75–89% of new hires), sometimes (50–74% of new hires), rarely (less than 50% of new hires), and I do not know or N/A.

Results: Of the potential 828 responses, 221 (27% overall response rate) were received. Responses were received from 163 (23% response rate) ASCLD and 58 (45% response rate) SOFT members. Responses from ASCLD and SOFT members were coalesced for analysis, with ASCLD members representing 74% of responses. Results for the most preferred educational background of entry-level candidates included 61 (36%) BS in physical or biological science and 46 (27%) MS in forensic science with a BS in physical or biological science. Results for the most preferred educational background for supervisory- or leadership-level candidates included 49 (29%) MS in forensic science with BS in physical or biological science.

Discussion: Results from this survey are consistent with works published in 1988 and 1996, despite the emergence of FEPAC accredited forensic science BS and MS programs. In this work, a BS in physical or biological science followed by a MS in forensic science was the most consistently preferred degree combination for both entry-level and supervisory-level candidates. This remained true when looking at candidate degree preference as a function of the respondents' terminal degree. There was a significant overlap between the respondents' own educational background and supervisory-level candidate preferences.

POSTER

P083 - Withdrawn

P084

A non-evaporative HLB dispersive pipette extraction (DPX) for sensitive analysis of therapeutic and abused drugs in urine

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Abstract

Introduction: Various extraction methods for drugs of abuse in urine have proven to be successful, including the use of HLB (hydrophilic–lipophilic balance) resin in traditional solid phase extraction. The HLB sorbent is extremely versatile in analyte binding because of the co-polymer phase containing both hydrophilic and lipophilic functional groups. In this poster we demonstrate a non-evaporative and highly sensitive extraction method for drugs of abuse in urine; the method gives a total of 10x dilution factor. With this new approach, a µXTR (micro-elution XTRaction) tip with 3 mg of Supel™ Swift HLB is utilized to extract 42 compounds across a large range of polarities. The method uses 50 µL of urine for minimal sample volume and 96 samples can be extracted in 15 minutes.

Objectives: Establish a robust, automated, and sensitive method for the analysis of therapeutic and abused drugs in urine.

Methods: The automated liquid handling (ALH) platform is a Hamilton MICROLAB® NIMBUS96. The DPX tips contain 3 mg of Supel™ Swift HLB sorbent (MilliporeSigma (Burlington, MA)). The sample is composed of 50 µL of urine fortified at various concentrations, 20 µL of B-One (Kura Biotech (Chile)), 100 µL of water, and 30 µL of internal standard (Cerilliant (Round Rock, TX)). Well plates for conditioning, wash, and elution steps are pre-aliquoted.

The DPX method begins with a two-step condition to ensure the HLB sorbent is properly wetted for binding; the ALH picks up the DPX tips and aspirates/dispenses 250 µL of 100% methanol and subsequently 250 µL of 100% water. The DPX tips are moved for sample binding, aspirating/dispensing the sample six times. The DPX tips are moved to aspirate/dispense 250 µL of water twice. Finally, the DPX tips are eluted by aspirating/dispensing 100 µL of 50/50 methanol/acetonitrile five times. The final dilution occurs by transferring 400 µL of water to the eluate, and the well plate is now ready for LC-MS/MS analysis. This protocol limits the percentage of organic to only 20% while also maintaining a minimal dilution factor. The method does not require solvent evaporation, unlike the majority of SPE methods currently used.

Analysis is performed on a Shimadzu LC40 paired with a SCIEX 6500+ mass spectrometer. The analytical LC column is a Restek Force Biphenyl, 3 µm, 50 x 2.1 mm LC Column (PN 9629352) paired with a Restek Force Biphenyl, 5 x 2.1 mm EXP Guard Cartridge (PN 962950252). The choice of a biphenyl column allows for optimal separation and more flexibility in sample composition for injection. Injection volume is 5 µL, and mobile phases are 0.1% formic acid in water and 100% methanol. This method was evaluated for linear dynamic range, extraction recovery, matrix effects, and limits of detection and quantification. Linearity is assessed by analyzing urine samples at 8 concentration points ranging from 1-500 ng/mL with n=3 at each calibration point replicated on three different days.

Results: Linear correlation coefficients for all analytes ranged from 0.995-1.00, and percent recoveries at 15 ng/mL ranged from 70-100% for most analytes measured (a total of 42 compounds were analyzed). Matrix effects were between between -31% to 21% (in this case, a negative value represents ion suppression while positive is ion enhancement). All analytes were quantified below the commonly used confirmation cutoff.

Discussion: The use of DPX µXTR HLB Tips for the analysis of drugs of abuse in urine is reproducible, sensitive, and fast. This method provides relevant LOQs with high throughput analysis with fast turnaround time for optimal efficiency in drug monitoring.

POSTER

P085

Systematic Web Monitoring of Drug Test Subversion Strategies in the United States

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Abstract

Introduction: In the US, employment related drug testing is widely used as a deterrent method to ensure a drug free workplace in both federally mandated programs and the general workforce. Given that negative drug tests are frequently a condition for employment, some recreational drug users try to subvert the testing. Many drug users discuss strategies for drug test subversion online, however, searching the internet for information about a specific topic is a daunting task given the sheer amount of information, and requires a systematic approach.

Objective: The aim of this study was to use systematic web monitoring to provide an overview of drug test subversion methods discussed by users.

Methods: Discussions about drug test subversions were analyzed from publicly available websites as well as from the dark web using the Researched Abuse, Diversion and Addiction-Related Surveillance (RADARS) System web monitoring program. Posts made in a six-month period between July 1 and December 31 of 2021 were collected from over 150 million websites worldwide (e.g., forums, blogs) utilizing a web-scraping tool. Posts of potential interest were identified through key words for inclusion. In addition, five exclusion terms were used to exclude irrelevant content. The keywords were selected based on previous studies of drug subversion and analysis of preliminary searches. The posts were manually coded to extract quantitative and qualitative information regarding drug test subversion tactics.

Results: In total, 634 relevant posts (566 public and 68 dark web) were included in the analysis. There was minimal difference in content between publicly available websites and dark web posts. Most posts discussed urine drug tests (85%), followed by hair (11%) and oral fluid (2%), and the most frequently discussed drugs were marijuana (72%) and cocaine (7.3%). The most common subversion tactic for urine drug testing was substitution of the specimen, either with a synthetic urine product or urine from a different individual. Another strategy was the use of a creatine supplement to mask a diluted urine. Posts regarding urine adulterants were rare. The main strategy suggested for hair test subversion was harsh treatment with products such as bleach, hair dyes, baking soda and/or laundry detergent. In addition, hair removal was discussed. Oral fluid subversion strategies focused on removing drug from the oral fluid cavity through vigorous brushing of teeth and tongue as well as the use of mouthwash, hydrogen peroxide, gum, and commercial detox products.

Discussion: While providing little evidence as to how effective the subversion strategies are, the study highlighted subversion strategies discussed online by donors. This information supports continued development of specimen validity testing to minimize the impact of drug test subversion attempts.

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P086

Modification of Specimen Validity Testing Ranges in Non-Regulated Urine Testing

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Abstract

Introduction: Testing for urine specimen acceptability has been termed by the National Laboratory Certification Program (NLCP) as Specimen Validity Testing (SVT). The national program requires laboratories to test for creatinine, pH, and at least one oxidizing adulterant (general oxidant) in addition to the drugs included in the federal panel. To determine whether a specimen is normal, dilute, or substituted, creatinine levels less than 20 mg/dL must be further tested for specific gravity by refractometer. Sample pH must be within the acceptable range of 4.5 to 9.0; specimens with values outside of this range must be realiquoted and analyzed by pH meter, and report as normal, invalid, or adulterated based on the confirmatory result. The general oxidant test was added to curb specimen adulteration by foreign substances or chemicals during collection.

Original SVT parameters, which were established using the limited data available at the time, are conservative and generate superfluous laboratory handling and testing. Repeated analysis for low creatinine values and recollection of urine samples based on elevated pH creates delays in reporting, ultimately reducing the number of job applicants that can be hired quickly. In cases of elevated pH, research supports that summer heat is the reason for higher urinary pH, not donor malfeasance. A dilute reporting status, which is generally insignificant to an employer seeking a new hire, requires additional specific gravity testing that delays the reporting of sample results.

Objective: Establish alternative SVT acceptance criteria by creating new acceptability ranges for creatinine and pH. Lowering the threshold for acceptable creatinine values and raising the levels of allowable pH would minimize sample follow-up testing and reduce unnecessary recollections that might otherwise lead to the denial of a job opportunity.

Methods: A retrospective analysis of hundreds of thousands of samples over two years' time was performed to identify trends in creatinine and pH values. Samples were collated by month to determine potential effects of seasonal temperature changes.

Results: Subsequent to the implementation of the adjusted upper limit of pH acceptability range, the number of specimens requiring additional testing for elevated pH was reduced by 61% over a single month. During summer months, data showed a dramatic increase in the number of specimens reported invalid due to pH greater than 9.0. In January of 2022, 0.14% of samples were reported invalid for elevated pH; that rate more than doubled in July of 2022, when samples reporting invalid due to high pH comprised 0.34% of total specimen volume. This seasonal increase is observed annually. By raising the maximum acceptable pH to 9.5, the proportion of samples reporting invalid for high pH is maintained around 0.05%, unaffected by seasonal changes in temperature or delays during transportation.

Lowering the minimum concentration of "normal" creatinine to 10 mg/dL from 20 mg/dL reduced the number of samples requiring specific gravity analysis by 80%. The monthly reduction rate has remained virtually constant and no substantial affect by seasonal difference in donor hydration is apparent.

Discussion: Alternative SVT criteria is limited to non-regulated urine testing and not applicable to the Federal Drug Testing Program. Because needs may vary by organization and industry, non-regulated substance abuse testing allows employers the customization of drugs and cutoffs in their panels. All SVT criteria changes were approved by clients prior to implementation, offering an additional way to individualize and improve testing programs.

POSTER

P087

Separation of Delta-8, Delta-9 THC and Chiral Hexahydrocannabinol metabolites in urine from Pain Management Compliance Testing

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Abstract

Introduction: The widespread legalization of recreational and medical marijuana has led to an increase in the use of cannabis and isolated cannabinoid analogs such as delta-8-tetrahydrocannabinol (delta-8-THC), which is an isomer of delta-9-tetrahydrocananbinol (delta-9-THC). These isolated psychoactive THC analogs, including delta-8-THC and hexahydrocannabinol (HHC), have similar pharmacological properties and have been rising significantly in popularity. The major THC analog metabolites in urine are ethers and ester glucuronide conjugates, which require hydrolysis prior to analysis in order to produce the free analytes of interest. Methods for chromatographic separation of these THC analog metabolites in urine are limited and chromatographically separating these metabolites has been a challenge.

Objective: To develop a liquid chromatography coupled to mass spectrometry (LCMSMS) method for the detection of delta-9-THC metabolites (11-OH Δ9-THC and 11-COOH Δ9-THC), Δ8-THC metabolites (11-OH D-8-THC and 11-COOH D-8-THC) and HHC metabolites (9R-COOH-HHC and 9S-COOH-HHC). The method will be used to detect these metabolites in authentic urine specimens.

Methods: A liquid chromatography-tandem mass spectrometry (UPLC-MSMS) assay was developed for simultaneous identification of THC analog metabolites (11-OH Δ9-THC, 11-COOH Δ9-THC 11-OH Δ8-THC, 11-COOH Δ8-THC, 9R-COOH-HHC and 9S-COOH-HHC) using commercially available deuterated internal standards (ISTD).

Samples were prepared by adding 10ng of internal standard to 100mcL of drug-free urine, calibrators (5-500ng/mL, controls (0, 15, 150 and 400ng/mL), or urine specimens. THC analog metabolites were hydrolyzed by alkaline hydrolysis (30mcL of 3N NaOH) and incubated at 58°C for 30 mins. Samples were cooled, acidified (30mcL glacial acetic acid) and extracted with 500mcL Hexane:Ethyl acetate (9:1) solution. Following mixing and centrifugation, 100mcL of the top organic layer was dried and reconstituted with 100mcL mobile phase solution (0.1% formic acid in water:methanol, 1:1) and placed in glass autosampler inserts. Analysis was performed using Waters AcQuity Xevo TQS-micro UPLC-MSMS system with a UCT SelectraCore® PFPP (2.1x50mmx2.7µm) column with guard column. The mobile phase components were: (A) 0.1% formic acid in water and (B) methanol. The injection volume was 5mcL. Flow rate was 0.3mL/minute; column temperature was 30°C. Total runtime was 9 minutes.

Authentic urine specimens received for pain management testing, which were previously screened for cannabinoids by immunoassay (n=82, 64 positive and 18 negative), were analyzed to determine the presence of these THC analog metabolites.

Results:

Respective retention times and MRM transitions:

11-OH Δ9-THC (5.8 min, 331>193, 200, 313)	11-COOH Δ9-THC (6.1 min, 345>299, 257 193)
11-OH Δ8-THC (5.6 min, 331>193, 200, 313)	11-COOH Δ8-THC (5.8 min, 345>299, 257. 193)
9R-COOH-HHC (5.7 min, 347>193, 207, 121)	9S-COOH-HHC (6.2 min, 347>213, 207, 121)

Chromatographic separation was achieved for the corresponding isomers (11-OH Δ9-THC and 11-OH Δ8-THC, 11-COOH Δ9-THC and 11-COOH Δ8-THC, and 9R-COOH-THC and 9S-COOH-HHC).

Of the 82 specimens analyzed, THC analog metabolites were only detected in the 64 specimens that screened positive: 11-COOH Δ9-THC (n=64, 5-5300ng/mL), 11-COOH Δ8-THC (n=20, 5-3590ng/mL), 9R-COOH-HHC (n=27, 6-134ng/mL),

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9S-COOH-HHC (n=26, 14-390ng/mL). Two metabolites (11-OH Δ9-THC and 11-OH Δ8-THC) were not detected in the tested specimens.

Conclusion/Discussion: **This method demonstrates the ability to separate and detect the THC analog metabolites of** delta-9-THC, delta-8-THC, and HHC. The authentic urine specimens tested contained predominantly 11-COOH Δ9-THC. While 11-COOH Δ8-THC was detected individually, it was more commonly detected in combination with 11-COOH Δ9-THC and HHC metabolites. HHC has only recently been reported in Europe but is now detected in urine samples in the US. With the increased legalization of recreational and medical cannabis/cannabis products, it is essential for laboratories to be able to separate and accurately identify delta-9-THC and delta-8-THC metabolites.

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POSTER

P088

Fentanyl and Acetyl Fentanyl Concentrations in Postmortem Blood Specimens over a Six Year Period

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Abstract

Introduction: At the end of 2016, Dallas County Southwestern Institute of Forensic Sciences (SWIFS) Toxicology Laboratory validated a new liquid chromatography-tandem mass spectrometry (LC-MS/MS) method for the detection and quantitation of cocaine, its metabolites, and select opiates, including fentanyl and acetyl fentanyl. Previously, the Toxicology Laboratory detected and reported these compounds qualitatively from gas chromatographic methods equipped with a mass spectrometer and flame ionization detector (GC-MS/FID). Additionally, in 2018, the Toxicology Laboratory validated a quadrupole time-of-flight (QTOF) drug screen, which replaced the comprehensive drug screen by GC-MS/FID. Since the implementation of these two more sensitive methods, the Toxicology Laboratory at SWIFS has seen an increase in fentanyl and acetyl fentanyl in postmortem specimens in the subsequent years.

Objectives: With the increased concern surrounding the use of fentanyl and its analogs and following the implementation of more sensitive instrumentation, SWIFS evaluated the quantitative data, demographical information, and medical examiner's cause of death from postmortem cases positive for fentanyl and/or acetyl fentanyl in blood specimens reported from the LC-MS/MS method from 2017 to 2022.

Methods: The quantitative results of fentanyl and acetyl fentanyl, demographical information, and medical examiner's determination of cause and manner of death were used to identify trends in the reporting of fentanyl and acetyl fentanyl, trends in concentrations over the six-year period, demographical changes, and the medical examiner's decision to include fentanyl and/or acetyl fentanyl in the cause of death.

Results: Between January 1, 2017, and December 31, 2022, fentanyl and acetyl fentanyl were identified in postmortem blood in 1,441 and 27 cases, respectively. Acetyl fentanyl was first reported from the LC-MS/MS method in 2018 with significantly lower number of positives when compared to fentanyl (n=2, n=122, respectively). While the number of fentanyl positive cases increased each year, acetyl fentanyl remained relatively consistent until 2022 when the number of acetyl fentanyl positive cases showed a five-fold increase from the previous year (n=3 in 2021, n=15 in 2022). The average postmortem blood concentration of fentanyl increased each year (7.1 ng/mL in 2018 to 11.7 ng/mL in 2022), whereas the average postmortem blood concentration of acetyl fentanyl was more variable with the highest average being in 2020 at 9.8 ng/mL. The largest increase in fentanyl positive cases was between 2020 (n=249) and 2021 (n=410). Fentanyl and/or acetyl fentanyl was most often identified in a white male in their late 30s. In the evaluation of the cause and manner of death, 74% of cases positive for fentanyl and/or acetyl fentanyl were ruled "Accidental," however, not all cases attributed the cause of death to these drugs. There was an increase in listing fentanyl as attributable to the cause of death from 20% of cases positive for fentanyl in 2018 to 74% of cases in 2022. Acetyl fentanyl was attributed to cause of death in 17 cases (63%).

Discussion: The growing public health concern with fentanyl has led to the proposal of legislation at the state (Texas House Bill 6) and federal (HALT Fentanyl Act) levels. Over the last six years, SWIFS has reported an increase in the number of cases positive for fentanyl and acetyl fentanyl. Additionally, medical examiners have more frequently attributed fentanyl to the cause of death in recent years. Further evaluation of data following any potential implementation of pending legislation should be assessed to determine if there is an impact on the prevalence of fentanyl in postmortem casework in Dallas County and surrounding areas and its attribution to the cause of death.

POSTER

P089

Fentanyl Overdoses and Use of Randox MultiSTAT as an Investigative Tool in Postmortem Toxicology

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Abstract

Background/Introduction: The opioid epidemic started in the early 1990s with the rise of prescription overdoses. In more recent years, heroin and fentanyl use has rapidly increased. Between 2019 and 2020, there was a 56% increase in overdose deaths caused by synthetic opioids, including fentanyl and fentanyl analogues. Fentanyl is 50 to 100 times more potent than morphine. The Randox MultiSTAT is an instrument used for presumptive testing purposes across multiple matrices. It has the ability to detect up to 21 drugs from a single sample in under 20 minutes.

Objectives: The first aim was to investigate fentanyl statistics in postmortem toxicology casework in 2022. The second aim was to evaluate the Randox MultiSTAT at the Alabama Department of Forensic Sciences (ADFS) Medical Examiner's Offices in order to triage whether a suspected overdose case receives a full or external autopsy.

Methods: Data for this project was mined from the ADFS Porter Lee Laboratory Information Management System (LIMS) from January 1, 2022, to December 31, 2022. The percentages of full versus external autopsies per region were calculated. Each case was screened for drugs using the Randox Evidence Analyzer. Positive samples were subjected to an extraction and then quantitated by liquid chromatography-mass spectrometry (LC/MS-MS). Both blood and oral fluid were qualitatively analyzed on the MultiSTAT. Confirmation specimens were collected for testing by enzyme immunoassay (EIA), LC/MS-MS, and gas chromatography-mass spectrometry (GC-MS). The analyses performed on the MultiSTAT were completed by the forensic pathology staff and the results were used for investigatory purposes by the medical examiners.

Results: In 2022, there were 1,832 overdose cases at ADFS. 1,304 (71%) were opioid overdoses. Fentanyl was found in 709 cases (39%). The median concentration was 13 ng/mL, while the maximum concentration was 403 ng/mL. Demographics for these cases included: 70% men, 30% women; 79% Caucasian, and 19% African American. The average age in these cases was 39 years old. In 2022, the ADFS Montgomery Medical Examiner's Office conducted 75% full autopsies and 25% external examinations only. Mobile Medical conducted 64% full autopsies and 36% external only. At Huntsville Medical, 96% of their autopsies were full, with only 4% being external only. To evaluate proof of concept, the MultiSTAT was first put into use at the Montgomery medical examiner's office. The remaining two medical examiner facilities will obtain MultiSTAT units later for additional studies. As of July 28, 2023, 91 cases were analyzed on the MultiSTAT at the Montgomery Medical Morgue. 45% of cases screened positive for fentanyl with the MultiSTAT within 20 minutes of autopsy completion. The screening cutoff concentration for fentanyl was 1 ng/mL. Using the MultiSTAT screen, the medical examiners chose to do 50 external examinations and 41 full autopsies. This increased the percentage of external examinations from 29% to 56%. Case types included suspected overdoses, child endangerment, and jail deaths.

Conclusion/Discussion: The use of fentanyl and fentanyl analogues has increased the number of overdose deaths not only in Alabama but in the rest of the United States. Fentanyl and fentanyl analogues are laced with other substances (e.g., xylazine) adding to the toxicity. Toxicology laboratories should strive to develop tools, workflows, and methodologies to combat the opioid epidemic such as using the Randox MultiSTAT as a tool during postmortem investigations.

POSTER

P090

Rapid, Simple, and Cost-Effective Postmortem Quantification of β-Hydroxybutyrate in Whole Blood Using the Abbot Precision Xtra® Point of Care Device

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Abstract

Introduction: β-hydroxybutyrate (BHB) is one of the three ketone bodies produced as a by-product of fat metabolism, primarily by liver acetyl coenzyme A, in fasting states when glucose is no longer available for cells to metabolize. Increased levels of this acidic anion leads to a drop in blood pH called (keto)acidosis. Biological fasting states resulting in ketoacidosis can be caused by diabetes mellitus (DKA), alcoholism (AKA), starvation, or hypothermia. Some publications indicate BHB is a better indicator than acetone to diagnose ketoacidosis.

The Abbott Precision Xtra® Meter is a handheld battery-operated device that performs individual measurements of BHB in whole blood samples using disposable single use capillary biochemical test strips. The Precision Xtra® Meter measures electrical current to determine the level of BHB in the sample. The current is produced when β-hydroxybutyrate dehydrogenase, which is present on the test strips, oxidizes BHB to acetoacetate. NAD+ is reduced to NADH, and a mediator molecule oxidizes the NADH back to NAD+, creating the proportional electrical current.

Objectives: Our objective was to improve the postmortem identification of DKA, AKA, starvation, or hypothermia in decedents by adding a method for rapid, simple, and cost-effective BHB determination.

Methods: Instrument verification of the Precision Xtra® Meter was evaluated with nine previously analyzed whole blood samples, identified with headspace GC/FID to be acetone positive or no acetone detected. The manufacturer supplied liquid quality controls (QC) low, mid, and high (Target concentrations of 0.6 mmol/L, 2.2 mmol/L, and 4.1 mmol/L), were included in the analysis, as well as a negative QC (ionized tap water). All samples and controls were analyzed per the manufacturer protocols without deviation. Refrigerated samples were allowed to come to room temperature and mixed by inversion prior to testing. The ketone test strip was inserted into the device, and a drop of whole blood was pipetted onto the strip. Results were returned in ten seconds with consistent, reliable performance and no instrumental errors were encountered. The expanded uncertainty of measurement was calculated to be +/-8.5% at the 95% confidence interval (k=2.074). Red-top, gray-top, and lavender top blood samples (n=3,10,4 respectively) were evaluated for suitability. The Precision Xtra® Meter cost $95 and each BHB test strip costs $4.55.

Results: As expected, blood samples with higher acetone levels using HS-GC/FID were associated with higher BHB levels using Precision Xtra® Meter, while low BHB levels corresponded with the acetone negative samples. During verification of device functionality per manufacturer instructions, it was observed that whole blood samples in red top tubes, without preservative or anti-coagulant, produced slightly higher, though not interpretatively different, BHB results than gray (sodium fluoride/potassium oxalate) or lavender (EDTA) collection tubes. Consistent results were found between gray and lavender type samples. Therefore, routine analysis was implemented in February 2023, to use blood sourced from gray and lavender-top tubes for BHB analyses. BHB testing was performed and tabulated on 88 cases using the Precision Xtra® Meter, received to the Franklin County Coroner's Office, with history of diabetes/abnormal biochemical findings (acetone, glucose, acetoacetic acid) from routine systematic toxicological analysis.

Discussion: BHB has been published to be stable in postmortem blood with no significant changes observed in relation to the postmortem interval. The authors found that BHB blood levels are useful, but should be interpreted in conjunction with blood acetone, vitreous glucose, and urine acetoacetic acid and urine glucose results, for the most accurate identification of ketoacidosis in postmortem cases. BHB blood levels greater than 2.5 mmol/L may be the most pathologically significant. This method provides rapid, simple, and cost-effective testing of decedents to supplement pathological findings of ketoacidosis.

POSTER

P091

Trends in Pediatric Intoxication Deaths in Maryland State Over a Twenty Year Period (2003 -2022)

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Abstract

Objectives: To conduct a retrospective toxicology results and demographic data review of pediatric intoxication deaths in Maryland over a 20-year period (January 2003- December 2022).

Methods: Retrospective state-wide data were collected from in-house Toxicology Laboratory results at the Office of the Chief Medical Examiner (OCME) in Baltimore, Maryland. The case database search included decedents with ages between 0 and 18 years and a cause of death which included exposure to one or more intoxicating substances. Cases were searched for case number, date of death, county of incident, age, sex, race, cause of death, manner of death, toxicology results, and brief case history. Data were analyzed for total numbers of drug, alcohol and toxic substance related pediatric intoxications, then subdivided by age, race, date of death, and major drug classes. The 38 distinct substances identified as contributing factors in pediatric intoxication deaths in Maryland database were categorized as ethanol, opioids, benzodiazepines, antidepressants, cocaine, antiepileptics, antihistamines, neuroleptics, carbon monoxide and other. Opioids were subdivided into fentanyl and analogs, methadone, oxycodone and morphine, among others, and evaluated by demographics and trends.

Results: From 2003 to 2022, a total of 157 fatal pediatric intoxications were autopsied at the MD OCME. In-house toxicology testing detected ethanol and 37 distinct drugs in blood and/or liver at time of death. Toxicology was positive for ethanol in 8 cases, and positive for other drugs in 155 cases. Pediatric intoxication fatalities ranged from 4 to 16 cases per year with an average of about 8 cases per year. Eighty (51%) decedents were identified as male, and ages ranged from about 2 months to 17 years. Female decedent ages ranges from about 2 months to 17 years. Seventy one (45%) decedents were age 10 or younger. Sixty nine decedents (44%) were identified as White, 54 (46%) as African American, 10 (6.3%) as Hispanic, 2 (1.3%) as Asian, with other subjects of unknown race. Prevalent substances involved in pediatric intoxication deaths included opioids (67%), carbon monoxide (12%), antidepressants (8%), antihistamines (8%) and benzodiazepines (7%). Manners of death included Undetermined (76; 2 months-17 years), Accident (38; 2 months-17 years), Homicide (26; 2 months-5 years) and Suicide (17; 12-17 years).

Of the 20-year total 157 fatal pediatric intoxications, the opioids category showed the highest incidence (106 cases). The opioids category showed a steady increase in incidence over time, from 1 case in 2003 to 15 cases in 2022. The most prevalent opioids were fentanyl and analogs (40 cases), methadone (32 cases), oxycodone (15 cases) and morphine (10 cases).

Discussion: Retrospective review of the last two decades of pediatric intoxication deaths in Maryland shows an increased incidence of opioid intoxications, likely related to fentanyl and analog related cases which increased from two in 2010 to eleven in 2022. Intoxication deaths occurred equally in males and females during the study period. The majority of decedents were identified as White or African American. The average decedent age was 8 years. Undetermined and Accidental manners of death were observed across the age range reviewed while Homicide as a manner of death was only observed in decedents five years and younger and Suicide as a manner of death was only observed in decedents twelve years and older.

POSTER

P092

Prevalence of Opioid Glucuronides in Human Hair

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Abstract

Introduction: Current hair testing methods, which rely on quantification of parent drug analytes, have difficulty distinguishing drug deposited into hair through physiological processes from drug deposited onto hair via external contamination. One approach to address this issue is to include analytes that are phase II conjugated metabolites because they are unlikely to be present through contamination.

Objective: In our previous work we developed a validated method for the quantification of opioid glucuronides in human hair and confirmed the presence of codeine-6-glucuronide, hydromorphone-3-glucuronide, morphine-3-glucuronide, morphine-6-glucuronide, and oxymorphone-3-glucuronide in opioid positive hair specimens. The objective of the work presented here was to determine relative concentrations of the glucuronides compared to parent drugs and determine the selectivity of these analytes as markers of opioid use by testing for these compounds in opioid positive and opioid negative hair.

Methods: Human hair was collected from volunteers and in partnership with a local substance abuse treatment center under IRB-approved protocols. 25mg of hair and 500 µL of M3 extraction reagent from Comedical (Trento, Italy) were placed in a heating block at 100 ˚C for 1 hour. The extraction solutions underwent solid phase extraction using Biotage Express CX extraction cartridges (3 cc/60 mg). The specimens were analyzed by liquid chromatography tandem mass spectrometry (LC-MS/MS) using multiple reaction monitoring. Samples were injected (5 µL) onto an Agilent Poroshell 120 SB-C18 (2.1x100 mm, 2.7 uM) column held at 40°C. Mobile phase A was 5 mM ammonium formate with 0.1% formic acid and mobile phase B was methanol with 0.1% formic acid. Limit of detection (LOD) for all glucuronides were 0.5 pg/mg, except morphine-6-glucuronide, which had an LOD of 0.25 pg/mg. LODs for codeine, oxycodone, and oxymorphone were 40 pg/mg and LODs for hydrocodone, hydromorphone, morphine, and oxymorphone were 80 pg/mg.

Results: A total of 97 hair specimens were analyzed. Of these, 31 had no detectable opioids and 66 had at least one opioid with a concentration greater than LOD. A specimen was considered positive for a parent drug if the concentration was at or above the Substance Abuse and Mental Health Administration (SAMHSA) proposed cutoff for opioids in hair of 200 pg/mg and positive for a glucuronide if the concentration was at or above 1 pg/mg. Of 33 morphine positive specimens, 82% were positive for morphine-3-glucuronide and 82% were positive for morphine-6-glucuronide. Of 6 codeine positive specimens, 100% were positive for codeine-6-glucuronide. Of 4 hydromorphone positive specimens, 75% were positive for hydromorphone-3-glucuronide. Of 6 oxymorphone positive specimens, 67% were positive for oxymorphone-3-glucuronide. Glucuronide concentrations as a percentage of parent drug concentrations ranged from 0.6% to 2.2%. No specimens that were below LOD for a particular parent had detectable glucuronides of that parent drug.

Discussion: This work demonstrates that opioid glucuronides are present at quantifiable concentrations in opioid positive hair specimens. Importantly, this work also demonstrates that hair with a parent opioid less than LOD (< 40 or 80 pg/mg) has no detectable corresponding glucuronides (< 0.25 or 0.5 pg/mg). Opioid glucuronides have promising potential to be markers for use that may permit differentiation of use versus external contamination in hair testing for opioids.

POSTER

P093

Running from a Truth: Lack of Regulation Promotes CBD in Health-conscious Markets Without Oversight

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Abstract

Introduction: Athletes have recently adopted the use of cannabidiol (CBD), as opposed to using non-steroidal anti-inflammatory drugs, for post-event treatment. CBD is marketed in the fitness industry to enhance muscle recovery, reduce inflammation and pain, and decrease cortisol levels, leading it to be one of the top three fastest growing natural products in the United States. Health claims and treatments for unapproved drugs are in violation of section 505(a) of the Federal Food, Drug, and Cosmetic Act, 21 U.S. Code §355(a). CBD is not an approved drug, with the exception of Epidiolex as treatment for certain seizure disorders. CBD is also not considered a dietary supplement by the Food and Drug Administration, which would exempt them from the U.S. Code requirement. The World Anti-Doping Agency and U.S. Anti-Doping Agency (USADA) ban the use of synthetic and phytocannabinoids but removed CBD from the lists in January 2019. USADA warns athletes to use natural products at their own risk. CBD remains on the list of banned substances for collegiate athletes under the National Collegiate Athletic Association.

Objectives: To analyze natural fitness products distributed to athletes at sporting events or through sports performance product subscription services for CBD content and truth-in-labelling.

Methods: The following products marketed towards fitness, including products distributed to runners after a half-marathon, were tested: Zen Energy Boost CBD strips, The After Bar Muscle Recovery Bar with CBD, River Organics CBD tincture, Zen Thermachill CBD sports cream, and EcoLip CBD lip balm. The products were extracted in 1:1 acetonitrile: water and agitated in a beadruptor for 1 min. A previously validated, published, and routinely used high performance liquid chromatography tandem mass spectrometry method was used for cannabinoid quantitation. Samples were analyzed using a Sciex ExionLC 2.0 liquid chromatograph attached to a Sciex 6500 QTRAP system with an IonDrive Turbo V source for TurbolonSpray. Analytes were separated on a Zorbax Eclipse XDB-C18 column with 10:90 water: methanol with 0.1 mM ammonium formate delivered at a flow rate of 1 mL/min. The following transitions were monitored in multiple reaction monitoring with: cannabichromene/Δ9-tetrahydrocannabinol (THC)/Δ8-THC/CBD 315>123 & 315>193; cannabinol 311>223 & 311> 241; cannabigerol 317>123 & 317>193; cannabidivarin/tetrahydrocannabivarin 287>165 & 287>231; CBDAA/THCAA 359>219 & 359> 341 and CBD-d3/THC-d3 318>123 & 318>193.

Results: Zen Energy CBD Boost strips with caffeine and Zen Thermachill sports cream were 26% and 12% above the labeled concentration of CBD, respectively. The After Bar was 57% below the labeled CBD concentration. The River Organics CBD tincture did not show information about CBD concentration and contained other cannabinoids, including Δ9-THC and cannabigerol. The Zen Energy strips also contained 0.3 mg/strip cannabigerol. The EcoLip balm contained less than 0.1 mg/lip balm of CBD.

Discussion: The unregulated CBD industry continues to infiltrate niche markets, making direct or word-of-mouth health claims with unsubstantiated authority. While data is emerging to support that CBD has some health benefits, reports also describe significant detrimental health effects such as liver damage and negative drug interactions. Health and fitness conscious individuals are led to believe these natural products are safer and healthier than other pharmaceuticals. Without industry oversight for quality products and a balanced message that consuming CBD may have significant health risks, consumers can become confused when faced with alternative realities of possible harm. The additional risk for an acute adverse event, with little-to-no accountability in the unregulated CBD marketplace, remains unconscionable. The unlabeled presence of THC in health and fitness products has significant repercussions for athletes who undergo substance testing in U.S. and international sporting events.

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POSTER

P094

An Advanced Method for Detecting Fentanyl, Fentalogues, Xylazine and Nitazenes by UH-PLC-MS/MS

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Abstract

Introduction: The incidence of new synthetic opioids (NSO) such as fentanyl analogues (fentalogues) has risen in toxicology casework. As tolerance of fentanyl and fentalogues appears to be steadily rising in these cases and structurally diverse NSO and sedatives such as nitazenes and xylazine continue being present in street fentanyl, toxicology laboratories must adapt their analytical methods.

Objectives: To advance the current Cuyahoga County Regional Forensic Science Laboratory's (CCRFSL) fentanyl and fentalogues method[1,2] by decreasing sample volume, extending dynamic ranges, and quantitatively reporting locally emerging NSO and select major metabolites.

Methods: The sample preparation consisted of osmotic lysing, protein precipitation with cold 10:90 methanol: acetonitrile, and centrifugation for ten minutes at 2,800 x*g*. The samples then underwent solid phase extraction using Phenomenex Strata® Screen-C 200 mg/6 mL columns conditioned with methanol and aqueous solution. The columns are rinsed with aqueous solution followed with methanol before elution with 78:20:2 methylene chloride: isopropanol: ammonium hydroxide. Instrumental analysis was performed with ultra-high performance Thermo Fisher Scientific™ Vanquish™ Horizon liquid chromatography system coupled to a Thermo Fisher Scientific™ TSQ AltisTM triple quadrupole tandem mass spectrometer (UHPLC-MS/MS) using a Kinetex® 2.6 µm F5 100 Å LC column (150 x 2.1 mm) at 40°C and 0.500 mL/min. The mobile phases were 0.1% formic acid in LC-MS grade water and acetonitrile.

Results: A seven-point linear calibration curve (1/x weighting) and quality control specimens were analyzed for 21 compounds. The quantitative compounds are norfentanyl, acetyl fentanyl, β-hydroxy fentanyl, fentanyl, carfentanil, o/m/p-fluorofentanyl, isotonitazene, butonitazene, metonitazene, protonitazene and xylazine. The qualitative compounds are norcarfentanil, 4-anilino-N-phenethylpiperidine (4-ANPP), *N*-desethyl isotonitazene, *N*-desethyl protonitazene, methoxyacetyl fentanyl, isovaleryl fentanyl, valeryl fentanyl, etodesnitazene, etonitazepyne and para-fluoroacetyl fentanyl. The separation of isomers such as isotonitazene and protonitazene and their metabolites and the inclusion of nitazenes and xylazine creates a compact method that was designed to meet quantitative reporting requirements for extracts.

Discussion: The significance of revising methods in laboratories is vital in these rapidly changing drug landscapes. Decreasing the necessary sample size allows for the analysis of low volume specimens to preserve critical evidence, increasing dynamic ranges reduces the number of repeat analyses with dilutions thereby reducing turnaround time, and quantifying locally relevant NSO provides data for future interpretive value.

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POSTER

P095

Detection of Phosphatidylethanol (PEth) in 12–15 Month-Old Whole Blood Specimens

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Abstract

Introduction: Phosphatidylethanol (PEth) is a group of phospholipids formed through phospholipase D-mediated enzymatic reaction between ethanol and phosphatidylcholine in cell membranes. PEth 16:0/18:1(16-carbon chain:0 double bonds/18-carbon chain:1-double bond) and16:0/18:2 (both commercially available) are the most abundant homologs found in whole blood. PEth is considered an alcohol biomarker and can provide information on the 'prolonged excessive exposure/consumption' in both social (half-life10-12 days) and heavy drinkers (half-life3-5 days). Distribution of PEth in blood has significant inter-individual variability attributed to genetics and lifestyle. A blood concentration PEth 16:0/18:1 ≥211ng/mL (300nmol/L) is indicative of alcohol abuse, while concentrations ≤21ng/mL (30nmol/L) is indicative of low alcohol exposure.

Objective: To identify and quantitate PEth (16:0/18:1 and 16:0/18:2) and evaluate 46 other PEth homologs in 12-15-month-old whole blood specimens submitted for alcohol testing.

Method: PEth 16:0/18:1 and 16:0/18:2 were quantitated, including the evaluation of 46 PEth homologs in whole blood using a modified previously published method. Briefly, 100µL of calibrators (5-5000ng/mL), controls (0, 15, 200 and 750ng/mL) and specimens were aliquoted into microcentrifuge tubes, combined with 25ng/mL PEth16:0/18:1-d5 (internal standard), 250ng/mL in H_2O:isopropanol (1:1), 100µL H_2O:acetonitrile (80:20) and mixed. Samples were loaded onto a Biotage ISOLUTE® SLE+400µL Sample Plate, and eluted with three-700µL aliquots of ethyl acetate:isopropanol (95:5). Eluates were dried and reconstituted with 100µL of isopropanol. Analysis was performed on a Waters TQS ultra-performance liquid chromatography-tandem mass spectrometry system, and Luna Phenyl-Hexyl column, (50 mm×3 mm×5µm). The mobile phase consisted of: A:2mM ammonium acetate and B:methanol/acetone (95/5). The method was validated in accordance with ANSI/ASB Standard 036 for linearity, precision, accuracy, selectivity, post-preparative stability, interference, carryover and limit of detection. Authentic de-identified whole blood specimens (n=65) submitted for alcohol testing (stored at 4-8°C for 12-15 months) were analyzed for 48 PEth homologs. Blood alcohol content (BAC) was determined using a validated headspace gas chromatography method.

Results: The assay was determined to be linear for PEth 16:0/18:1 and 16:0/18:2 from 5-5000ng/mL with an r2 > 0.9990(n=3); accuracy was within ± 20% (n=15) with intra (n=5) and inter-run precision (n=15) of <13% CV. Analytes were stable for 24 hours post-preparation. No interferences were observed. PEth concentrations for calibrators and controls were stable over the validation testing period.

Ethanol positive authentic blood samples (n=44) (BAC=0.01-0.31%w/v) had results for PEth 16:0/18:1 (83-4074ng/mL), 16:0/18:2 (88-2270ng/mL) and 32 additional homologs were detected. PEth 16:0/18:1 and 16:0/18:2 were determined in 10 samples to be <211ng/mL (29-197ng/mL). Ethanol was not detected in 21 samples; 12 of which were negative for PEth, 9 were positive for PEth 16:0/18:1 (<20-131ng/mL) and 16:0/18:2 (<20-110ng/mL) along with 8 additional homologs. 14 of the homologs were not detected in any samples.

Discussion/Conclusion: The presented method for the determination of PEth 16:0/18:1 and 16:0/18:2 is robust, sensitive and selective. No change in BAC concentrations over the 12-15-month storage period was observed. PEth was detected in some BAC negative samples at concentrations <211ng/mL which is not indicative of alcohol abuse. BAC positives samples with PEth <211ng/mL(n=10), also not indicative of alcohol abuse. The presence of PEth in negative BAC samples demonstrates alcohol exposure and does not indicate impairment. This is the first report the authors are aware of showing PEth detection beyond 60 days refrigerated storage. This study demonstrates that PEth's most abundant homologs are still detectable in whole blood after 12 months under refrigerated conditions and that an isolated measurable PEth concentration should be interpreted with caution in determination of impairment status.

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POSTER

P096

National Laboratory Certification Program Studies in the Preparation of Hair PT Samples

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Abstract

Introduction: In 2023, the National Laboratory Certification Program (NLCP) at RTI International (RTI) undertook a program to develop Proficiency Testing (PT) samples for drugs in hair. Most drug challenges will be authentic donor hair, but in some situations suitable donors will be difficult to find. In such cases, mainly regarding rare analytes (e.g., phencyclidine, PCP and methylenedioxymethamphetamine, MDMA), the drugs must be spiked into the hair. Unfortunately, current spiking protocols cannot be used if the samples are to be decontaminated prior to analysis, as some, or all, of the spiked drugs will be lost. Therefore, a method must be developed to spike drugs into hair that results in drugs being resistant to removal by decontamination.

Objectives: The objective of this study was to develop an in vitro spiking protocol for PCP (used as model substance) capable of producing samples that can withstand decontamination that behave similarly to donor hair. These spiked samples must be intact strands without extensive damage.

Methods: A negative hair "pool" was prepared by combining hair from three donors and mixing in water for one hour. After drying overnight, the hair was weighed into 24 aliquots of about 100 mg each. To spike, the aliquots were soaked in a solution of phencyclidine (PCP) at 20 µg/mL or 2 µg/mL. The solutions were prepared in either methanol, methanol with 1% formic acid, Comedical® M3® reagent, or a modified phosphate-buffered saline (PBS) solution with each spike performed in triplicate. The samples were placed on a shaker for two hours, then removed from the solution and left to dry for four weeks. Next, the samples were subjected to an extended phosphate buffer decontamination, dried for 48 hours, and submitted for testing.

Results: The modified PBS solution resulted in the highest uptake of drug. This was followed by methanol at about 30 times less uptake than the PBS. Methanol with 1% formic acid and the M3® reagent showed minimal uptake of drug.

Sample Number	PCP Solution	PCP Results (pg/mg) (CV)
12666-128-1	20 µg/mL methanol	298 ± 53.3 (18%)
12666-128-2	2 µg/mL methanol	<40
12666-128-3	20 µg/mL methanol, 1% formic acid	<40
12666-128-4	2 µg/mL methanol, 1% formic acid	Not Detected
12666-128-5	20 µg/mL M3® Reagent	Not Detected
12666-128-6	2 µg/mL M3® Reagent	Not Detected
12666-128-7	20 µg/mL Modified PBS	11,990 ± 1.344 (11%)
12666-128-8	2 µg/mL Modified PBS	1,121 ± 51.4 (5%)

Note: Reference laboratory's Limit of Quantification is 40 pg/mg

Conclusion/Discussion: When hair is soaked in water, it swells by an average of 31% in as little as 15 minutes. Swelling increases in cases where the hair is more porous and may be as high as 50% for very porous hair. Although methanol is a swelling solvent for hair, the time required for swelling in methanol is greater than water. Methanol containing acid is swelling but it is also an extractive solution which might explain the lower concentration of drug detected when formic acid was added. M3® reagent is used to extract drugs from hair. The inclusion of the M3® reagent was to determine if it was effective in promoting drug uptake.

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Modified PBS solution was shown to produce spiked hair with a high concentration of PCP likely due to the rapid swelling of the hair with the aqueous solution. Furthermore, utilizing this spiking process, the drug was resistant to decontamination after drying the hair for an extended time. Future work includes scaling up this process and testing the ability to target specific concentrations of PCP in the spiked hair.

P097

4-Year Evaluation of Drug Impaired Driving Drug Concentrations

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Abstract

Introduction: Impaired driving is an on-going public health concern in the United States. Recently, there has been an increased focus on drug detection in impaired driving along with concomitant use of alcohol. Through the efforts of the National Safety Council's Alcohol Drugs and Impairment Division (NSC-ADID) scope recommendations and cutoff levels, impaired driving data has improved. However, one area that remains relatively unexplored is the evaluation of changing patterns in terms of both drug concentration and drug positivity for all Tier I drugs.

Objectives: The objective of this research was to review drug concentration data for NSC-ADID Tier I drugs over a four-year period (2017-2020) to assess fluctuations in drug concentrations over time as well as evaluate overall drug positivity rates using a large data set.

Methods: Data used was compiled from reported results from suspected DUID cases received by NMS Labs between 2017 to 2020. Average, median, maximum, and minimum concentrations were calculated for each drug. A Z-test (α=0.05) was performed to determine the difference in positivity for each Tier I drug year to year; an F-test (α=0.05) was used to determine if the variance in drug concentrations were different year to year. Using the F-test results, a T-test with either equal or unequal variance was employed to determine if the average concentration for each drug changed year over year.

Results: The total number of cases per year is provided in Table 1.

Table 1. Number of cases per year

Year	No. of Cases Submitted
2017	17,346
2018	17,471
2019	19,050
2020	16,539

*1,840 (10%) cases from 2020 not included.

The most identified drug class was cannabinoids, with approximately 50% of the cases each year having a cannabinoid positive finding. In terms of individual drugs identified in the cases over the four years, delta-9-THC and its metabolites, ethanol, amphetamine/methamphetamine, fentanyl, and alprazolam were the top five drugs. Positivity for delta-9-THC and ethanol can be found in Table 2 along with positivity rates for cases where delta-9-THC and ethanol were found in combination, which was the most frequently encountered drug combination. Positivity rates for delta-9-THC, amphetamine, methamphetamine, and fentanyl showed all statistically significant increases over the four years.

Table 2. Positivity of delta-9-THC and ethanol.

Drug	2017	2018	2019	2020
Delta-9-THC	45%	46%	46%	49%
Ethanol	59%	59%	61%	53%
Delta-9-THC + Ethanol	18%	19%	20%	20%

Related to drug concentrations, the general trend was that the average concentrations were relatively stable over time

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for most drugs. Exceptions to this included a statistically significant increase in the average concentration of fentanyl (5.7 ng/mL in 2017 to 9.6 ng/mL in 2020), methamphetamine (301 ng/mL in 2017 to 381 ng/mL in 2020), and delta-9-THC (6.4 ng/mL in 2017 to 7.3ng/mL in 2020). Other findings included increases in the maximum reported concentrations between 2017 to 2020 for amphetamine (1,400 to 2,700ng/mL), methamphetamine (5,550 to 13,000ng/mL) and fentanyl (56 to 310 ng/mL). Statistically significant concentration decreases were noted for several CNS depressants and many narcotic analgesics other than fentanyl.

Discussion: There were observable differences in both drug positivity and average concentration for all classes of drugs evaluated. Increases in positivity were observed with the cannabinoid, CNS stimulant, and narcotic analgesic drug classes, while CNS depressants declined in positivity. The drugs with the largest increases in positivity over the four years were delta-9-THC, fentanyl, amphetamine, and methamphetamine. Notable changes in concentrations were observed for several drugs in Tier I. Laboratories should consider the impact of changing drug concentrations to ensure methods have adequate sensitivity and/or methods are appropriately validated for dilution to bring high concentrations within range.

P098

Towards Determination of Recent Cannabis Use: Optimizing Cannabinoid Recovery from an Impaction Filter Device

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Abstract

Introduction: The Applied Chemicals and Materials Division and the Statistical Engineering Division at the National Institute of Standards and Technology are investigating methods for non-invasive, breath-based determination of recent cannabis use. As part of this effort, breath samples from cannabis users during acute cannabis elimination will be collected with an impaction filter device and analyzed offline for Δ9-tetrahydrocannabinol (THC), cannabidiol (CBD), and cannabinol (CBN). Prior to this data collection, a "processing" (analyte extraction and preparation) protocol is needed that ensures consistent handling and favorable specifications for the extraction of cannabinoids. Many choices are available in the extraction and preparation of cannabinoids from impaction filter devices prior to analysis. For the essential steps of the protocol, choices must be made, for example, about extraction solvent and vial material(s). Choices must also be made about whether to include discretionary steps, such as including a "keeper" additive (e.g., ethylene glycol) or sonicating the filters. These discretionary steps add time and labor to the procedure. The ideal protocol would specify choices that facilitate recovery of the desired compounds, while excluding laborious practices that offer little improvement. To develop an effective and efficient protocol, the effects of various processing choices were explored through a principled designed experiment.

Objectives: In support of developing a streamlined protocol for a forthcoming human study, the goal of this work was to identify processing choices that influence the recovery of THC, CBD, and CBN from one impaction filter device. The primary objective was to determine the choices and settings that improve cannabinoid extraction, with a secondary objective being to quantify improvement in recovery due to influential discretionary steps.

Methods: A three-part experiment was conducted to identify factors affecting cannabinoid recovery from polypropylene impaction filters. The processing choices explored in this experiment included vial material, vacuum concentrator temperature, keeper, solvent, cannabinoid concentration, and sonication. Blank breath samples were collected from a non-cannabis user and individual filters were spiked with 0.3 or 3 ng per filter, representative of authentic breath samples. These were extracted and processed prior to quantitative analysis by liquid chromatography with triple quadrupole mass spectrometry. Processing settings were determined using principled multi-factor experiment design methodology to efficiently screen factors and estimate main and interaction effect sizes. The design of each sequential experimental section was directed by the insights from the previous section, allowing successive installments to expand upon and corroborate the experimental results.

Results: Results from this experiment identified the processing choices that significantly influence the recovery of THC, CBD, and CBN from spiked breath samples. These include vial material (glass) and addition of a keeper (ethylene glycol or octanol). Other choices, such as elution solvent and sonication, were found to offer no improvement in the recovery. There were large differences in the average recovery rate between the three cannabinoids that were independent of factor settings, with CBD having the lowest and CBN the highest recovery, with variability in recovery consistent across compounds. The effects of the active factors were consistent across compounds, simplifying protocol adoption for optimizing cannabinoid extraction from breath samples.

Discussion: Principled sequential experimentation was used to identify extraction and preparation choices that affect recovery of THC, CBD, and CBN from the impaction filters within a breath aerosol sampling device. While this research was limited to spiked breath samples, it is reasonable to expect the general conformance of results to extend to breath samples collected during acute cannabis elimination. Thus, the results from this work were used to develop an efficient, evidence-based processing protocol foundational to a larger research effort dedicated to the development of a breath-based methodology for determination of recent cannabis use.

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P099

Designer Benzodiazepines in Alabama Over a 5-Year Period

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Abstract

Introduction: Designer benzodiazepines (DBZD) are a class of drugs similar to classic benzodiazepines (BZD) such as alprazolam, clonazepam, lorazepam, etc., but with the important difference that DBZD are not legally prescribed or sold in the United States. DBZD include metabolites and structural analogues of other BZD; some of these are legally prescribed in other countries, but are sold illegally in the U.S. Both groups are considered sedative-hypnotics and have a profound effect on the central nervous system (CNS) by binding to the GABA receptors in the brain.

As CNS depressants, DBZD have a high potential for abuse and are classified as Schedule IV drugs. Commonly prescribed BZD are used to treat anxiety, panic disorders, and sleep disorders. Side effects include drowsiness, dizziness, and fatigue. Both classical BZD and DZBD are abused for their sedating effects which can alleviate the "crash" phase of stimulants as well as extend the euphoric effects of opioids.

At the Alabama Department of Forensic Sciences (ADFS), 28 BZD and DBZD are routinely analyzed. Of those 28 drugs, 19 of them are DZBD. Over the last five years, ADFS has seen an uptick in the prevalence of DBZD. Some have moved through the Novel Psychoactive Substance (NPS) market quickly without gaining popularity in Alabama, while others have been seen for several years. The newest DBZD to appear in Alabama is bromazolam, and it has gained popularity very quickly.

Objectives: To investigate the prevalence of designer benzodiazepines in cases analyzed by ADFS from 2018-2022 to highlight the most common drugs, and to identify user demographics. To investigate bromazolam prevalence in 2023, and to identify user demographics.

Methods: Death cases and Driving Under the Influence of Ethanol/Drugs (DUID) cases were screened by enzyme-linked immunoassay using the Randox Evidence Plus Analyzer. Qualitative confirmation of DBZD was performed by liquid-liquid extraction with Agilent Triple-quadrupole LC-MS/MS. Microsoft Excel was used to filter and sort the data.

Results: From 2018-2022, ADFS reported 476 confirmed DBZD results in both DUID and death cases. The most prevalent targets were clonazolam, etizolam, flubromazolam, flualprazolam, and, with totals of 72, 72, 115, and 177, respectively, accounting for 92% of the total DBZD cases. There were 294 DUID cases, three child endangerment cases, one sexual assault case, one assault victim case, and 140 death cases. User demographics were 58% male, 42% female, 79% White, 17% Black, 2% Hispanic, and 2% listed as Asian or other.

Bromazolam was confirmed in 70 cases from January 2023 through May 2023, representing 79% of the total DBZD cases for that period. Of those cases, 32 were DUID cases and 38 were death cases. User demographics for this drug were 66% male, 34% female, 70% White, 29% Black, and 1% Hispanic.

Discussion: Designer benzodiazepines are commonly abused drugs in Alabama. More DBZD are detected in cases involving living people than in death cases. Etizolam and bromazolam had a similar number of cases in both living and deceased users, suggesting that these two DBZD are more dangerous than other DBZD and classical BZD. Flualprazolam was the most reported DBZD by ADFS from 2018-2022, but has tapered off since 2020. Clonazolam peaked in 2021, but prevalence has decreased since then. Flubromazolam surged in number of cases in 2019, dipped significantly in 2020, only to resurge again in 2021 before dipping again in 2022, indicating its strong hold on the NPS market.

Bromazolam was added to the scope of analysis at ADFS in late 2022 and has quickly become the most prevalent DBZD in Alabama with a total of 73 cases. If this trend continues, it may become the most reported DBZD in Alabama.

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P100

Drug Evaluation and Classification (DEC) cases in Ontario: A 2-year review of regional trends and toxicology results

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Abstract

Introduction: Ontario is the most populated province in Canada (>15 million people) and drug impaired driving has become a public safety issue for citizens. The Drug Evaluation and Classification (DEC) Program was instituted in 2008 by the Federal government, and it gives drug recognition evaluators (DRE) the ability to demand biological samples in suspected drug impaired driving investigations. Police involved in these cases in Ontario submit biological samples to the Centre of Forensic Sciences (CFS) for toxicology testing which involves general screening and multi-targeted methods which can detect and/or quantitate prevalent prescription, over-the-counter and illicit drugs including novel psychoactive substances.

Objectives: To present information on toxicology findings in drug-impaired driving investigations as well as the DRE's opinion on drug class and compare findings regionally.

Methods: A retrospective analysis of drug impaired driving cases was performed from January 1, 2021 and December 31, 2022. Data pertaining to the investigation, location of arrest, accused demographics, the DRE opinion on drug class, sample type, and toxicological drug findings were reviewed.

Results: There were 2211 and 1228 DEC urine and 806 and 653 DEC blood cases that underwent toxicological examination in 2021 and 2022, respectively. Driver demographics, DRE opinions and drug prevalence in blood/serum and urine will be analyzed for regional differences and presented.

Discussion: The number of suspected impaired driving cases submitted for toxicological analysis decreased from 2021 to 2022. The distribution of the DRE opinion on the drug category(ies) involved, drug findings in relation to location and driver demographics, as well as drug prevalence and trends will be identified and presented.

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P101

A Postmortem Case Report Involving Desalkylgidazepam and Bromazolam

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Abstract

Background/Introduction: Novel psychoactive substances (NPS) pose a great threat to society due to many factors including the lack of regulation and limited information regarding the pharmacological and toxicological effects of NPS in humans. Designer benzodiazepines are a class of NPS that have been detected worldwide contributing to impaired driving, drug-facilitated crimes, and nonfatal and fatal overdoses. Two such benzodiazepines, desalkylgidazepam and bromazolam, have recently emerged and their prevalence has been internationally reported.

Objective: The objective of the study was to analyze desalkylgidazepam and bromazolam in postmortem specimens using gas chromatography coupled with mass spectrometry (GC-MS).

Case Summary: A 24 year-old white male with history of drug abuse, traumatic brain injury, and depression was found deceased surrounded by drug paraphernalia. Upon investigation by the Sheriff's Office seized drug laboratory, desalkylgidazepam was presumptively identified in the yellow-powdery substance that was found at the scene.

Methods: Desalkylgidazepam and bromazolam were tentatively identified in blood and urine by gas chromatography-nitrogen phosphorous detection (GC-NPD), and then confirmed by gas chromatography-mass spectrometry (GC-MS), which prompted further investigation. A fit-for-purpose validation study was performed to add desalkylgidazepam and bromazolam to the previously validated methods for traditional benzodiazepines. Parameters including precision, bias, linearity, and limit of quantitation were assessed.

For desalkylgidazepam, the samples were processed by addition of internal standard (desalkylgidazepam-d5) and phosphate buffer followed by centrifugation. Solid-phase extraction (SPE) was performed which included conditioning with methanol, water, and phosphate buffer, washing, and elution with methylene chloride:isopropyl alcohol: ammonium hydroxide (78:20:2, v/v/v). Derivatization was achieved with N-methyl-N-(tert-butyldimethylsilyl)-trifluoroacetamide with 1% tert-butyldimethylchlorosilane (MTBSTFA). The tert-butyldimethylsilyl derivatives were identified by GC-MS operated in selected ion monitoring mode (desalkylgidazepam: m/z 371, 427, and 357; desalkylgidazepam-d5: m/z 378 and 433).

For bromazolam, the samples were processed by addition of internal standard (bromazolam-d5) and phosphate buffer, followed by centrifugation. SPE was performed which included conditioning with methanol, water, and phosphate buffer, washing, and elution with methylene chloride: isopropyl alcohol:ammonium hydroxide (78:20:2, v/v/v), and final reconstitution in methanol. Bromazolam and its internal standard were identified by GC-MS operated in selected ion monitoring mode (bromazolam: m/z 325, 352, and 353; bromazolam-d5: m/z 330 and 357).

Results: The fit-for-purpose laboratory validation studies met method criteria and were deemed acceptable. The linear range for desalkylgidazepam and bromazolam were 50-1000 ng/mL and 5.0-100 ng/mL, respectively. The results of the analyses were as follows:

	Desalkylgidazepam (ng/mL)	Bromazolam (ng/mL)
Postmortem blood	1100	352
Postmortem urine	89	398

Additionally, fentanyl (11 ng/mL) was detected in the blood and fentanyl, norfentanyl, and gabapentin were detected in the urine.

Conclusion/Discussion: GC-MS analysis of the postmortem blood and urine samples confirmed the presence of desalkylgidazepam and bromazolam. The co-detection of fentanyl and designer benzodiazepines has been prevalent in the illicit

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drug community and has been reported as “Benzo Dope”. The bromazolam concentration reported in this case report is higher than previous literature reports. There are limited reports on desalkylgidazepam concentrations from illicit use. Given their increased prevalence, these analytical methods will assist in characterization and analysis of such NPS benzodiazepines in future casework.

P102

Analysis of Xylazine, Opioids, and Other Common Adulterants in Blood and Urine by SPE and LC-MS/MS

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Abstract

Introduction: Xylazine is a veterinary sedative emerging as a popular adulterant in the illicit drug market. It is often seen with powders and tablets containing fentanyl. This combination of drugs is commonly referred to as "Tranq". Although xylazine is not an opioid, its use with fentanyl is having a significant impact on the opioid epidemic for a number of reasons. It can induce a state of unconsciousness, worsen addiction, potentially increase the risk of fatal overdose, and its effects are not counteracted by naloxone. According to the DEA, in 2022, xylazine was detected in 48 out of 50 states. Of all of the fentanyl-positive samples tested by the DEA, 23% of powder samples and 7% of tablet samples also contained xylazine. The trends of xylazine usage parallel to fentanyl, indicating that it is likely to persist. This poster details a robust and effective method for the simultaneous analysis of fentanyl, fentanyl analogs, xylazine, and other common adulterants by SPE and LC-MS/MS.

Objectives: Develop an LC-MS/MS method capable of separating (para-, ortho-, and meta-) fluorofentanyl isomers and create a solid phase extraction procedure to simultaneously analyze fentanyl, fentanyl analogs, xylazine, and other common adulterants.

Methods: UCT's Clean Screen® DAU cartridges were utilized for extraction. Samples were prepared at low, medium, and high concentrations of 5, 25, and 80 ng/mL from a stock solution standard mix. Urine samples were prepared by adding 1 mL of sample, 0.5 mL of methanol (MeOH), and 2.5 mL of phosphate buffer (pH 6.0, 0.1 M). Blood samples were prepared by diluting 0.5 mL of sample in 3 mL of phosphate buffer, followed by mixing and centrifugation. DAU cartridges were conditioned with 3 mL of MeOH and equilibrated with 3 mL of water and 3 mL of phosphate buffer. The samples were loaded onto the cartridge and washed with 3 mL of 100 mM HCl followed by 3 mL of MeOH. The target analytes were eluted with 3 mL of 2% ammonium hydroxide in MeOH. After evaporating, the extracts were reconstituted in 1 mL of 5% MeOH in water.

Samples were analyzed on SelectraCore® DA UHPLC column (100 mm x 2.1 mm, 2.7 µm) using a Shimadzu Nexera LC-30AD coupled with MS-8050. Mobile phase A consisted of 0.1% formic acid in water and mobile phase B was MeOH. All analytes were analyzed in positive mode.

Results: Analytes were successfully extracted from urine and blood with high recoveries and low matrix effects at low, medium, and high concentrations of 5, 25, and 80 ng/mL (n=3). Extraction recoveries of analytes from urine ranged from 88-119%. Aside from acepromazine, which was not the focus of this panel, relative standard deviations (RSDs) were ≤ 20% and matrix effects were within ± 25%. Extraction recoveries from blood ranged between 80-113%. With the exception of acepromazine, relative standard deviations (RSDs) were ≤ 20% and matrix effects were within ± 25%. For this method, the LOD is 0.5 ng/mL, and the LOQ is 1 ng/mL.

Discussion: An optimized LC-MS/MS method utilizing the SelectraCore® DA UHPLC column was developed that enabled efficient resolution of (para-, ortho-, and meta-) fluorofentanyl isomers in a short, 12-minute total run time. Fentanyl, para-fluorofentanyl, xylazine, and the other eight additional drugs included in the panel were successfully extracted from urine and blood using the Clean Screen® DAU cartridges with excellent recoveries, matrix effects, and RSDs. These results indicate that this optimized method is highly efficient, can be readily implemented in high-throughput laboratories, and that xylazine and other adulterants can easily be incorporated into existing fentanyl panels.

Disclosure

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

Conflict of Interest Salary

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P103

Review of first year LC-QTOF-MS screening of blood specimens from suspected DUID cases for NPS Benzodiazepines

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Abstract

Introduction: Prior to 2020, the North Carolina State Crime Laboratory (NCSCL) Toxicology section used a combination of a 12 panel ELISA and a Basic Solid Phase Extraction (BSPE) analyzed by Gas Chromatography – Mass Spectrometry (GC-MS) to screen suspected DUID cases. Starting at the end of 2019, the GCMS screen was replaced with a 220-drug panel Liquid Chromatography – Quadrupole Time-of-Flight Mass Spectrometer (LC-QTOF-MS) screen. The initial panel only contained a handful of Novel Psychoactive Substances (NPS) Benzodiazepines that had been previously identified in casework. Subsequent additions to the QTOF panel have included several NPS Benzodiazepines that were not detectable using the prior testing protocol. This presentation will discuss the results of a retrospective analysis for NPS Benzodiazepines in first year casework following the incorporation of a QTOF screen in DUID casework.

Objective: The objective of this study is to review the results of a retrospective analysis for NPS Benzodiazepines in suspected DUID casework submitted to the NCSCL.

Methods: Suspected DUID cases submitted to the NCSCL during 2020 were retrospectively examined for NPS Benzodiazepines that were not part of the initial testing.

Initial screening of samples by Immunoassay was done using Immunalysis™ Benzodiazepine Direct ELISA kit. Following this, samples were extracted by protein precipitation using cold 50:50 methanol: acetonitrile followed by centrifugation. The supernatants were dried down using Nitrogen and reconstituted in 80:20 water: methanol. The samples were then injected onto a SCIEX ExionLC™ AD system coupled to a X500R QTOF. The LC and QTOF analytical parameters were set to those outlined in the SCIEX vMethod™.

The LC-QTOF-MS method was validated following ANSI/ASB Standard 036. The validation included interferences, from both matrix and other drugs, carryover assessments, limit of detections (LOD), and a concordance study.

The results of the retrospective analysis were reviewed and compiled to determine the prevalence of NPS Benzodiazepines in NCSCL DUID casework.

Results: In 2020, NCSCL received 5,106 DUID case submissions. The initial QTOF method targeted only Diclazepam, Etizolam, Flubromazepam, Flubromazolam, and Phenazepam as these NPS Benzodiazepines were previously detected in DUID casework. In early 2021, Flualprazolam was added to the QTOF panel after increasing prevalence in cases. However, case monitoring revealed an increasing number of unconfirmed indicative Benzodiazepine immunoassay results by either the QTOF panel or GCMS analysis. In the 4th Quarter of 2021, Clonazolam, 8-aminoclonazolam, and Bromazolam were added to the QTOF panel. However, the BSPE with GCMS analysis did not provide the same level of sensitivity for these compounds. Due to a backlog of cases some of these 2020 cases were analyzed after the additions of the newer NPS Benzodiazepines. Immediately following the addition of these compounds to our QTOF panel our unconfirmed Benzodiazepine assays decreased and 8-Aminoclonazolam became our third most prevalent NPS in 2020 after Flualprazolam and Etizolam. Retrospective analysis of QTOF data from cases where immunoassay testing indicated Benzodiazepines revealed an increase in prevalence especially for 8-Aminoclonazolam.

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	Bromazolam	Clonazolam	8-Aminoclonazolam
Initial	3	9	173
Retrospective	6	19	310
% Increase	100	111.1	79.2

Heat maps generated from the retrospective analysis mirror those of 2020 NCSCL DUID submissions.

Conclusion/Discussion: Including LC-QTOF-MS screening into DUID casework has allowed for seamless addition of emerging drugs. This is especially important as most legacy GCMS methods don't afford the sensitivity needed to identify emerging drugs. As seen with 8-Aminoclonazolam, the need to monitor metabolites may be more informative about drug prevalence than monitoring of the parent alone. Additionally, the heat maps provide insight into the continuous need to monitor emerging drug trends especially from jurisdictions with large DUID submissions.

P104

Analysis of Δ^8 and Δ^9 THC Metabolites and Other Cannabinoids by SPE and LC-MS/MS

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Abstract

Introduction: In recent years, there has been a notable increase in the use and commercialization of Δ^8-THC products. This increase stems from the passing of the Farm Bill of 2018, which legalized hemp. A prominent cannabinoid of hemp is cannabidiol (CBD) which can be converted into Δ^8-THC. This federally legal alternative cannabinoid poses another complication for states where recreational cannabis is illegal. Due to the isomeric nature of Δ^8 and Δ^9-THC, chromatographic separation of the parent drugs is critical. However, THC is extensively metabolized by the body into 11-hydroxy-THC and 11-carboxy-THC. Therefore, the chromatographic separation of the metabolites of Δ^8 and Δ^9 THC is of interest to many laboratories. 11-hydroxy-Δ^8-THC and 11-hydroxy-Δ^9-THC are exceptionally challenging to separate using liquid chromatography.

As new alternative cannabinoid products continue to emerge, this leads to further identification and separation challenges for laboratories. New cannabinoids of interest include THC isomers Δ^{10} and $\Delta^{6a,10a}$, THC homologs tetrahydrocannabiphorol (THC-P) and tetrahydrobutocannabutol (THCB), as well as several others. These examples are included in the panel with other emerging cannabinoids.

Objectives: Develop an analytical method that can separate Δ^8 and Δ^9-THC metabolites and other cannabinoids of interest. Introduce an effective solid-phase extraction (SPE) method for the extraction of cannabinoids from urine.

Methods: Urine sample extractions utilized Styre Screen® HLB 60 mg, 6 mL SPE columns. For sample preparation, 1 mL of sample, internal standard(s), 1 mL of acetonitrile, and 1 mL of 100 mM phosphate buffer pH 7.0 were added to a test tube and vortexed. Hydrolysis was not performed for this research. SPE columns were conditioned with 3 mL of methanol (MeOH) and 3 mL of 100 mM phosphate buffer, pH 7.0. After loading the sample onto the column and aspirating at 1 to 2 mL/minute, the samples were washed with 3 mL of deionized water (DI H_2O) and 3 mL of 40:60 MeOH:DI H_2O. SPE columns were dried under full pressure for at least 10 minutes before eluting with 3 mL of 60:40 MeOH:Hexane. Lastly, samples were evaporated at 10 psi, 40°C, and reconstituted in 1 mL of initial mobile phase composition 45:55 MeOH:-DI H_2O. Samples were analyzed using a Shimadzu Nexera LC-30 AD with MS-8050 equipped with a SelectraCore® PFPP (pentafluorophenylpropyl) column (100 x 2.1 mm, 2.7 µm) and a guard column (5 x 2.1 mm, 2.7 µm). Mobile phase A was 5 mM ammonium formate with 0.1% formic acid in deionized water and mobile phase B was methanol. The extraction procedure's recovery, matrix effect, and relative standard deviation were evaluated across three concentrations.

Results: Separation of Δ^8 and Δ^9-THC, their metabolites, and other cannabinoids was achieved on LC-MS/MS using UCT's SelectraCore® PFPP column. This diverse column offers alternative selectivity to C18 allowing efficient separation of difficult isomers, 11-hydroxy-Δ^8-THC and 11-hydroxy-Δ^9-THC. Urine samples were evaluated at three concentrations 5, 25, and 50 ng/mL. Using a pre and post-spike technique, all sample recoveries were calculated to be above 75% with a range of 78-118%. Matrix effects were assessed by comparing post-spiked samples and solvent standards which were within ± 25%. Lastly, relative standard deviations were calculated to be all less than 20%.

Discussion: This poster outlines an analytical method that addresses a common problem faced by many forensic laboratories that perform cannabinoid analysis. While C18 is a popular phase for analytical column, due to the non-polar nature of cannabinoids, PFPP proved to be more effective in separating the 11-hydroxy-THC isomers. The poster further emphasizes the effectiveness of a robust sample preparation technique for urine extractions, which in turn aids in reducing instrument downtime and maintenance requirements.

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P105

Screening and Confirmatory Testing of a Counterfeit M-30 Pill Adulterated with Xylazine

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Abstract

Introduction: Criminal drug networks are mass-producing fake pills and falsely marketing them as legitimate prescription pills to deceive the unsuspecting customers. Fake prescription pills are easily accessible and often sold on social media and e-commerce platforms, making them available to anyone with a smartphone, including minors. More recently, xylazine, a veterinary tranquilizer, has been reported as an adulterant in an increasing number of illicit drug mixtures, and has been linked to a growing number of overdose deaths. The presence of xylazine in counterfeit M-30 pills has been a disturbing trend in drug seizures for the past two years across the United States.

Objectives: This method will illustrate the ability to quickly screen counterfeit material from a customizable target list of drugs (library) using an LC-TOF instrument to facilitate the identification of unidentified compounds and demonstrate the use of quantitative and confirmation analyses using an LC-MS/MS tandem quadrupole system.

Methods: The M-30 pill extract, provided by San Diego County Sheriff's Department Regional Crime Laboratory, was prepared using a simple dissolve, filter, dilute and shoot technique. The seized drug screening experiment utilized full scan accurate mass data with fragmentation, which facilitated compound identification by comparing to a pre-existing high resolution library with 400 commonly abused drugs. An ACQUITY™ RDa™ Detector was used for the screening analysis and compounds separated using a UPLC™ system gradient with buffered ammonium formate and acetonitrile over 7 minutes on a ACQUITY HSS T3 2.1 x 100 mm column.

Confirmatory testing by LC-MS/MS was performed for positive identification of the screened analytes by Waters. Using an additional 100 fold dilution of the sample, a Xevo™ TQ Absolute tandem quadrupole mass spectrometer, column and mobile phase was used for the relative quantitative analysis with a fast 2.5min gradient elution profile.

Results: The screening method identified fourteen presumptive positive compounds, including xylazine, in the counterfeit M-30 pill based on a comparison of expected and observed retention time, accurate mass and instrument intensity response. The observed mass for xylazine was m/z 221.1110 with a mass error of 1.3 ppm (0.3 mDa), and the observed retention time was 2.30 min compared to the expected 2.38 min.

Confirmatory testing using a relative quantitative experiment was performed for eleven of the fourteen identified compounds due to availability. Quantitative and qualitative MRM transitions were used for each compound. Fentanyl was detected at a concentration of 7444 ng/mL and xylazine at 912 ng/mL. 4-Methylamino antipyrine, 4-ANPP, O(p)-fluorofentanyl, acetaminophen and caffeine were also detected at levels above 500 ng/mL.

Discussion: An example of a screening and confirmation workflow has been demonstrated for the analysis of a counterfeit M-30 pill adulterated with xylazine. The workflow follows identification recommendations from the Organization of Scientific Area Committees (OSAC) on seized drug analysis utilizing Categories A and B techniques to provide the highest level of selectivity through structural information and chemical and physical characteristics.

LC-TOF provides a quick and efficient method to perform a comprehensive screen on seized drug samples. In this example a complex mixture of fourteen compounds, in a pill that should contain one active ingredient, were presumptively identified. The combination of accurate mass, short analysis time, customizable and expandable library and a full scan data acquisition with fragmentation, provides a high confidence result for the drug screening experiment which facilitates retrospective data analysis when a result is not obtained from the target library search. Confirmation testing using LC-MS/MS provided retention time and MRM relative quantitative data for the compounds identified. Xylazine was detected and quantified at 912 ng/mL in the counterfeit M-30 pill.

POSTER

P106

2-Methyl AP-237: A Case Series involving a Novel Synthetic Opioid

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Abstract

Introduction: 2-Methyl AP-237, sometimes colloquially referred to as 2MAP, is a novel psychoactive substance (NPS) with opioid agonist activity that was recently detected in the illicit drug market. 2-Methyl AP-237 is an analogue of bucinnazine or AP-237, a substance once used in China for the treatment of chronic pain in cancer patients but never medically used in the United States. In vitro studies show mu-opioid receptor binding at a lower percentage than fentanyl or morphine and potency approximately 4 times higher than morphine. It is categorized as a cinnamylpiperazine, which is structurally distinct from fentanyl and its analogues, therefore evading the domestic class-wide scheduling action on fentanyl-related substances enacted by the Drug Enforcement Administration (DEA) in February 2018. Because of this circumvention of the law and fueled by the opioid epidemic, substances like 2-methyl AP-237 and its structural isomer AP-238 were discovered in drug seizures. 2-Methyl AP-237 was first identified in the drug market in 2019; the DEA issued an alert in 2021 to inform the public about this emerging substance

Objectives: Toxicological information about 2-methyl AP-237 is limited at this time so assessing 2-methyl AP-237's toxicological role in a death investigation is challenging, especially in the context of poly-substance use. The objective of this presentation is to review postmortem casework where 2-methyl AP-237 was reported.

Methods: Potential 2-methyl AP-237 findings were identified through routine casework by Liquid Chromatography/Time-of-Flight Mass Spectrometry (LC/TOF-MS) and the use of a surveillance library, or discussions with submitting agencies regarding case histories. 2-methyl AP-237 was added to the surveillance library in April 2022. Upon review of the finding based on accurate mass, retention time, chromatographic shape, and mass error, additional testing could be suggested in particular cases. Confirmatory analysis was performed upon request using Liquid Chromatography/Tandem Mass Spectrometry (LC-MS/MS) to separate structural isomers and with standard addition for quantitation. Trend information was compiled and co-positivity with other drug findings was evaluated where available.

Results: Between 2021 and 2022, seven postmortem blood cases were positively identified and quantitated for 2-methyl AP-237. Concentrations of 2-methyl AP-237 ranged from 11-320 ng/mL with an average and median concentrations of 202 ± 146 ng/mL and 310 ng/mL, respectively. Cases tested were received from AZ, MI, PA, SC, TX, WA, and WI, with one case from each state indicating no regional trend for 2-methyl AP-237. Demographics for these cases include all male users, ages 20-51. Six of the seven cases included comprehensive toxicology testing, with all six finding at least one benzodiazepine present; these benzodiazepines were predominantly categorized as NPS, i.e. delorazepam, 8-aminoclonazolam, flualprazolam, etizolam/alpha-hydroxyetizolam. Other NPS reported in these cases included alpha-PHP/alpha-PiHP, phenibut, and mitragynine.

Discussion: 2-methyl AP-237 is a novel synthetic opioid (NSO) that is not structurally related to fentanyl. Because it circumvents current scheduling actions focused on fentanyl-related substances and nitazene-related substances (another subclass of NSO), the cinnamylpiperazines are compounds of concern and therefore should be monitored in toxicological assays. Though rarely encountered, when combined with other substances, it can lead to toxicity and death. Many of these cases reported combinations with benzodiazepines, and "benzo-dope" formulations can pose higher risk of overdose to users due to enhanced central nervous system depression. The identification of this drug indicates a change in illicit manufacturing to use non-fentanyl opioid sources to circumvent legislative bans. The death investigation community should be aware of compounds like this when a suspected opioid death turns up with insignificant findings, and toxicology labs should have resources available to test for the presence of compounds like 2-methyl AP-237.

POSTER

P107

LC-MS/MS-based Quantification of 47 Therapeutic Drug Monitoring Compounds in Serum: A Simple Sample Preparation Strategy for Efficient Analysis

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Abstract

Introduction: Liquid chromatography with tandem mass spectrometry (LC-MS/MS) methods are considered the gold standard for therapeutic drug monitoring (TDM) of medications. TDM involves measuring drug levels in a patient's blood to ensure optimal dosing and maximize efficacy while minimizing side effects. LC-MS/MS provides several advantages for TDM, including high sensitivity, selectivity, and the ability to analyze multiple drugs in a single sample. Typically, serum or whole blood are the standard matrices used for TDM, but serum is often preferred due to its ease of handling compared to whole blood. However, serum is a complex biological matrix containing various endogenous compounds that can interfere with analysis. These matrix effects may affect the accuracy and precision of drug concentration measurements, often requiring difficult sample preparation methods to be used. In this study, 11 anticonvulsants, 22 antidepressants, and 14 antipsychotics were spiked into serum and extracted using a Phree protein precipitation plate before being analyzed on a SCIEX QTRAP 6500+ system. Recoveries from the samples prepared using Phree were then compared to the recoveries of samples prepared without phospholipid removal.

Methods: A total of 47 TDM compounds were spiked into blank serum from UTAK at concentrations of 1 ng/mL, 5 ng/mL, and 50 ng/mL. Then, 100 µL aliquots were added to protein precipitation plates with an oleophobic membrane and a sorbent to selectively remove phospholipids (Phenomenex Phree). Protein precipitation began upon the addition of 300 µL acetonitrile, followed by gentle shaking for 5 minutes before elution and collection of the filtrate on a Preston positive pressure manifold. Control samples were prepared at a concentration of 50 ng/mL in blank serum, and 100 µL aliquots were protein precipitated with the addition of 300 µL acetonitrile in a centrifuge tube. The supernatant was then filtered into autosampler vials through a 0.2 µm filter.

Liquid chromatography was performed using a Shimadzu LC-Nexera at a flow rate of 0.3 mL/min with a Phenomenex Kinetex 2.6 µm Biphenyl, 100 x 2.1 mm column. Mobile phase A consisted of HPLC grade water with 0.1% formic acid and 5 mmol ammonium formate. Mobile phase B was a 50:50 mixture of HPLC grade methanol and acetonitrile with 0.1% formic acid and 5 mmol ammonium formate. Mobile phase B was ramped from 10% to 30% over 0.1 min, then brought to 50% at 3 min. Mobile phase B was further ramped to 100% at 5 min and held for 2 min. Samples were then injected into the 6500+ QTRAP system for MRM analysis.

Results: The Phree™ PLR extraction successfully removed phospholipids from serum samples when compared to protein precipitation alone. This resulted in reduced ion suppression and improved detection of the target analytes. External calibration curves without internal standards were constructed using a regression model of 1/x, showing good linearity with R^2 values ≥ 0.991 from 0.5 -100 ng/mL for all analytes.

Percent recovery ranged between 70% and 130% for all compounds, except for dehydroaripiprazole and mirtazapine, which fell below 70%. Average concentrations for extraction replicates (n=3) were calculated based on 1 ng/mL, 5 ng/mL, and 50 ng/mL spikes. Mirtazapine consistently showed lower calculated concentrations compared to the spiked samples. The addition of internal standards would improve concentration accuracy.

Conclusion: In conclusion, this study demonstrated the effectiveness of a simple sample preparation strategy for the low-level quantification of 47 TDM compounds in serum using LC-MS/MS. The comparison of samples prepared with and without phospholipid removal demonstrated the effectiveness of the Phree sorbent in eliminating matrix effects caused by phospholipids, thus improving the accuracy and precision of drug concentration measurements.

POSTER

P108

The use of Cocaine Hydroxy Metabolites in a Decision Model for the Interpretation of Ingestion vs. Exposure in ChildGuard® Samples

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Abstract

Introduction: Hair testing is a reliable, noninvasive, and established biological matrix for toxicological drug of abuse testing (Boubma etal. 2006). There is a demand for reliable tools to differentiate between ingestion and exposure in hair analysis. Cocaine is a commonly abused substance and is a routinely tested for substance. A decision tree using cocaine hydroxy metabolites in hair has been published (Scholz et al. 2019) to assist in the determination of ingestion or contamination. This decision tree and others like it are all based on specimens that have been washed prior to analysis. ChildGuard® (available up to a 14 panel) testing is designed to detect passive exposure to drugs, distinguishing between both parent drugs and drug metabolites in hair specimens, and the specimens are not washed prior to analysis. We present a specific decision model based on a high risk population of hair specimens that are not washed prior to analysis.

Objective: The objective of this study is to develop a decision model for ingestion vs exposure using cocaine hydroxy metabolites for our unwashed ChildGuard testing sample types.

Methods: Keratinized specimens (hair or nail) were weighed out (50 mg). Hair is collected from the vertex area and nails are typically fingernails. The whole hair or nail sample was homogenized in a Biospec Beadbeater-24. The specimens were incubated at 53° C in 0.1 M hydrochloric acid for a minimum of two hours. The sample was centrifuged and the supernatants were subjected to a solid phase extraction procedure and eluted with Methylene Chloride:Isopropanol:Ammonium Hydroxyide (80:18:2). The samples dried under nitrgen at 40 degrees C. The dried extracts were reconstituted in 0.1% formic acid in deionized water prior to analysis by a Agilent 1200 Liquid Chromatography Sciex 5500 Tandem Mass Spectrometry (LC-MS/MS). The following analytes are included in the method: cocaine, benzoylecgonine, norcocaine, cocaethylene, o-hydroxycocaine, and p-hydroxycocaine.

Results: Samples received from May 2022 to March of 2023 were included in this study (n=260). All specimens with a cocaine concentration below the laboratory cutoff (100 pg/mg) were excluded from the study. The age range of the data set was from newborn to age 47.5, with a mean of 5.9 and a median of 3.1. Ratios from a separate internal cohort (n=21) of known users was used to set the criteria for the model. The next criterion was the ratio of Norcocaine/Cocaine ≥ 2% and the ratio of a hydroxycocaine/cocaine ≥ 0.1%. Samples that were less than these criteria would be classified as suspected exposure and equal to or greater than would be classified as suspected ingestion. This decision model was then applied to our data set of keratinized specimens yielding two outcomes, individuals with suspected ingestion (n=25) and individuals with suspected exposure (n=235). We did not distinguish between hair and nail as all keratinized were included.

Discussion/conclusion: Various criteria for interpretation of cocaine ingestion vs exposure in hair have been proposed. This study used Scholz et al. as the starting point for the model. The difference between the Scholz model and this model is due to the lack of removing external contamination through a decontamination wash step in this study. The inclusion criteria for the Scholz et al. test is cocaine ≥ 500 pg/mg compared to our criteria of cocaine ≥ 100 pg/mg. This identified an additional 6 samples as consistent with suspected exposure. The inclusion of the metabolic ratios show that 188 samples would have an agreed upon outcome between the two models. The remaining 66 samples would have their determination changed from ingestion (Scholz) to exposure (USDTL), due to not having a removal of external contamination.

POSTER

P109

The emergence and implications of altered codeine content in poppy seeds used in the food industry

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Abstract

Introduction: On February 17, 2023, the US Department of Defense (DoD) issued a memorandum cautioning service members that consumption of poppy seeds could produce a positive urinalysis result for codeine above the current cutoff of 2,000 ng/mL. Emerging evidence indicates that new varieties of *Papaver somniferum* L. can contain varying amounts of alkaloids, including food-grade varieties with reduced alkaloid content and pharmaceutical-grade varieties that contain high amounts of a particular alkaloid with one variety bred specifically to produce codeine. Poppy seeds from pharmaceutical-grade plants are often sold to the food industry in the US as by-products, although washing and extended thermal treatment have been shown to reduce alkaloid contamination by 50-80%. There is currently no regulation limiting codeine or morphine content in poppy seeds used in the food products in the US with the amount of codeine reportedly ranging from 0-320 µg/g and the amount of morphine ranging from 15-200 µg/g in poppy seeds, confounding differentiation between poppy seed consumption and opioid abuse.

Objectives: The codeine and morphine content of different brands of poppy seeds were determined, including a brand that has been reported to produce opiate-positive urinalysis results exceeding the DoD cutoff.

Methods: Three different poppy seed brands were purchased from chain stores or found in food products in their bakery departments. Poppy seeds from each source (500 mg) were pulverized using a 2010 Geno/Grinder. Three separate 50-mg measurements of pulverized seeds were placed in tubes, and 2 mL of HPLC-grade methanol was added. After mixing for 15 min, the top layer of methanol was decanted and dried in a TurboVap at 40°C under nitrogen. The dried extract was reconstituted in 1 mL methanol, and 100 mL was diluted with 300 mL ISTD and 400 mL 95:5 formic acid mobile phase. These extracts were then analyzed for codeine and morphine by LC-MS/MS.

Results: Two of three poppy seed brands had high levels of codeine with the third brand having no detectable codeine (mean±SD: 280.18±30.73; 227.36±1.84; 0 µg/g). The morphine concentration was low in all three brands (mean±SD: 5.31±1.25; 0.49±0.01; 1.61±0.12 µg/g, respectively).

Discussion: The seeds with the highest codeine level (280.18 µg/g) were acquired from the same product as those evaluated in a recent study in which some subjects had urinary codeine concentrations exceeding 2,000 ng/mL within 4-6 hours of poppy seed consumption (Lewis et al, Pain Medicine, 22:2776–2778, 2021). Because this recent evidence supports the possibility that codeine-positive urine tests could result from poppy seed ingestion, the ability to differentiate between use of a legal food product and codeine abuse is becoming more challenging and might require changing certain regulations to ensure that urinalysis does not lead to an accusation of drug abuse following consumption of a legal food product.

POSTER

P110

Development and Validation of an LC-MS-MS Method for the Quantitative Analysis of Ten Cannabinoids

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Abstract

Introduction: *Cannabis sativa* (*C. sativa*) has been used for seed and fiber, medicine, for religious ceremonies, and for recreational purposes for years. Cannabinoids are the main constituents of the plant and are present in different ratios in the various chemovars. The dramatic increase in cannabis legalization resulting in the proliferation of many cannabis based products has led to increased demand for accurate and reliable analysis methods to quantitate relevant cannabinoids.

Objectives: The objective of this project was to develop and validate a rapid and sensitive liquid chromatography-tandem mass spectrometry method for the determination of the ten major cannabinoids in cannabis plant material and cannabis-related products available on the market. These cannabinoids were cannabidivarin (CBDV), cannabidiol (CBD), cannabigerol (CBG), delta-9-tetrahydrocannabivarin (THCV), delta-9-tetrahydrocannabinolic acid-A (THCAA), cannabidiolic acid (CBDA), cannabinol (CBN), cannabichromene (CBC), delta-9-tetrahydrocannabinol (Δ^9-THC), and delta-8-tetrahydrocannabinol (Δ^8-THC).

Methods: Method optimization was evaluated using cannabinoids' reference standards to optimize sample injection, chromatographic resolution, source ionization, and data acquisition. An Applied Biosystems/MSD Sciex (ABSciex) QTRAP 3200 LC-MS/MS system with a Shimadzu Prominence HPLC was used. The system utilized a dual pump, a vacuum solvent micro-degasser, a controlled-temperature autosampler, and an MS-MS detector with a turbo-ion ESI source operating in the positive-ion multiple reaction monitoring (MRM) mode. The LC-MS/MS analysis was conducted using a Raptor ARC-18 (150 mm x 4.6 mm ID x 2.7 µm) column with a Raptor ARC-18 EXP guard column cartridge maintained at 28 °C. MRM transitions were monitored for each compound for maximum selectivity and sensitivity. The resulting MS/MS fragment spectra were consolidated in AnalystTM 1.6.3 software (SCIEX). A gradient mobile phase consisting of 0.1 % formic acid in water (mobile phase A) and 0.1 % formic acid in acetonitrile: methanol (75: 25) (mobile phase B) was used at a flow rate of 1.2 mL/min. The LC gradient was 78% B at the start, 95% B at 16 min, and 78% B at 19.10 min, and stopped at 19.50 min. Three internal standards were used for the quantitation of the target cannabinoids, including (D3-CBN), (D3-CBD), (D3-Δ^9-THC). D3-CBD was used for the quantitation of CBDV, CBD, CBDA, CBG, and CBC, while D3-Δ^9-THC was used for the quantitation of THCV, THCAA, Δ^9-THC, and Δ^8-THC, in addition to D3-CBN which was used for the quantitation of CBN. The working range of the validated method was 50– 2500 ng absolute for all the target cannabinoids. The proposed method was validated using the reference solutions at three levels: low, medium, and high according to the ICH guidelines with respect to linearity and range, the limit of detection (LOD), the limit of quantification (LOQ), specificity, precision, and accuracy. Samples of cannabis products and plant material were prepared by taking known amounts either by dissolving or extracting with methanol, spiked with internal standards, vortexed, and analyzed.

Results: The method showed good linearity as demonstrated by regression values $R2 \geq 0.99$ over a range of 50 ng to 2500 ng absolute levels for all the target cannabinoids. The method showed excellent sensitivity with a LOD of 15 ng absolute, while the LOQ was 50 ng absolute. The intra-day precision (%RSD) ranged from 0.53% -11.30%, while the inter-day precision ranged from 1.11%-11.46%. The method showed good accuracy calculated as % recovery which ranged from 88%-114%.

Conclusion/Discussion: With the rapid growth of cannabis legalization either for medicinal or recreational purposes, it is crucial to develop and validate new analytical methods. Using the current successfully validated LC-MS/MS method, ten major cannabinoids can be easily identified and quantified in different cannabis plant samples and cannabis products widely available on the market.

POSTER

P111

Sensitive and accurate characterization of Z drugs, metabolites and their degradation profile in urine using LC-MS/MS

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Abstract

Objectives: Non-benzodiazepine sedative-hypnotics, colloquially referred to as "Z drugs", are a new class of sedative drugs that are structurally different than benzodiazepine but share similar mechanisms of drug action. In addition to their sleep-inducing effects, this class of drugs presents potential risks and adverse side effects such as nausea, drowsiness, dizziness, varying degrees of consciousness, and in extreme cases, coma, and respiratory failure. In this study, a rapid, robust, and sensitive method for the detection and quantification of common Z drugs (zolpidem, zolpidem phenyl-4-carboxylic acid, zaleplon, zopiclone, and N-desmethyl zopiclone) and their metabolites in human urine is described. Their degradation profile under different conditions was also investigated using a nominal mass triple quadrupole and QTOF instruments. The combination of the two systems enabled accurate and sensitive quantification of Z drugs and metabolites while providing confident identification of the degradation by-products.

Methods: Calibrator solutions of the 5 Z drugs in the study were prepared in concentrations ranging from 1 to 2000 ng/mL in synthetic urine. Samples were analyzed using a dilute and shoot approach. The spiked calibrator solutions were diluted 10-fold by diluting the 100 µL of blank synthetic urine with 10% methanol with 0.1% formic acid in water to a final volume of 1 mL. HPLC separation was performed on an ExionLC system using a Phenomenex Phenyl-Hexyl column. Mobile phases used consisted of ammonium formate, methanol, and appropriate additives. The injection volume was 5 µL. Data were collected using a SCIEX 5500+ system for quantitative analysis and SCIEX X500B QTOF System for qualitative characterization of the metabolites and additional degradation products. Both instruments were operated with electrospray ionization (ESI) in positive mode. Data was acquired on the SCIEX X500B QTOF System using independent data acquisition (IDA).

Results and Discussion: Chromatographic separation of the analytes was accomplished using a 5-minute LC runtime, which was sufficient to achieve near baseline separation of all the Z drugs in the panel. The series of nine calibrator solutions displayed excellent linearity over the calibration range, with R2 values greater than 0.99 for each of the five analytes. Method precision was evaluated at the three lowest calibrator levels (1, 2.5, and 5 ng/mL) for each of the 5 Z drugs in the study. The %CV values ranged from 0.75 to 11.65%, demonstrating the robustness of the method at the low end of the calibration range. Accuracies for the spiked urine samples ranged from 80 to 100% demonstrating excellent robustness and quantification performance.

The degradation profile of zopiclone was investigated using both the SCIEX 5500+ system and the SCIEX X500B QTOF system. Zopiclone spiked in synthetic urine was subjected to five different solvent conditions over the course of 14 hours in a chilled HPLC autosampler kept at 8 ºC. Degradation of zopiclone was observed under neutral and weakly basic conditions. However, zopiclone appears to be stable under acidic conditions. These results suggest that the rapid degradation of zopiclone can be alleviated with the addition of acid.

The degradation profile of zopiclone and conversion to 2-amino-5-chloropyridine (ACP) was further investigated using the acquired TOF-MS/MS spectra on the QTOF. IDA generated comprehensive and high-quality MS/MS spectra, enabling accurate and confident identification of ACP as a degradation by-product of zopiclone in urine by using library spectral matching. SCIEX OS Software confirmed the identification of ACP using mass error, retention time, isotopic distribution, and MS/MS spectral library matching. The results highlight the use of the X500B QTOF System as a powerful tool for reliable and confident compound identification through MS/MS spectral library matching.

POSTER

P112

Phenibut Concentrations in a Fatality Quantitated using Method of Standard Addition

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Abstract

Introduction: Phenibut, a $GABA_B$ receptor agonist, is a psychotropic drug. Synthesized in Russia in the 1960's, it's primarily used to treat anxiety, insomnia and alcohol withdrawal. In North America, phenibut is unavailable by prescription, but is easily imported through internet suppliers. It's illegal for products within the USA to contain phenibut, but its use remains unrestricted in both Canada and the USA. Despite being marketed as a "natural" product, neither country recognizes phenibut as a nutritional supplement. Individuals who attempt self-medication may inadvertently overuse, misuse, experience tolerance and withdrawal symptoms. Phenibut toxicity/withdrawal may have overlapping symptoms including hallucinations, delirium, muscle spasms, tachycardia or respiratory depression. There have been no fatalities documented solely from phenibut use.

Objectives: Phenibut was detected in a case specimen during a routine forensic drug screen using LC-Accurate Mass Spectrometry. The objective of this study was to develop a confirmatory method using Method of Standard Addition (MSA) to quantify phenibut in post-mortem whole blood.

Methods: A 30-year-old male, found deceased is his locked Winnipeg apartment, screened positive for phenibut. The specimen was quantitated using a validated MSA by LC-ESI-MS/MS, which confirmed the presence of this compound. Three MRM transitions were identified for phenibut following infusion of an analytical reference standard (Cayman Chemical, P/N 9002652; ≥98%): 180.1 m/z (parent ion) and 145.2, 91.2 and 117.5 m/z (product ions). Chromatography was accomplished using an Allure PFP Propyl, 50 x 2.1mm, 5.0µm analytical column beginning with 90% MP_A (Aqueous) and 10% MP_B (Acetonitrile), where both mobile MP's contain 0.2% formic acid and 2mM ammonium formate, then ramping to 10% MP_A:90% MP_B over 10 mins at 0.5 mL/min. Gabapentin-d10 was used as the internal standard.

Results: A targeted forensic drug screen conducted on a whole blood specimen having undergone protein precipitation was positive for naproxen, acetaminophen, 7-aminoclonazepam, fluoxetine/norfluoxetine and phenibut. Phenibut has not been detected in case work in Manitoba to date, and as this analyte is not routinely quantitated in most forensic toxicology laboratories, confirmation was achieved using MSA. Phenibut eluted at 1.88 min and appeared as a symmetric, resolved peak in all transitions. The assay was validated following ANSI/ASB Standard 054 (2021). Linearity (quadratic,$1/x^2$: 1 to 50 µg/mL, r^2 0.9998), accuracy (% bias < 10% at 4 and 45 µg/mL), carryover (none observed), limit of detection (0.1 µg/mL), dilution integrity (1 in 3, 1.80% CV) and interferences (none observed) were evaluated. Quality controls at 4 and 45 µg/mL were prepared using a different source of phenibut (TRC, P/N A630595) than the calibrators. The calculated phenibut concentration in the case specimen was 29 µg/mL.

Discussion: The effects of phenibut consumed recreationally are felt 2-4 hours post-ingestion. Unfortunately, this lag time is often misconstrued in naive users as an ineffective dose. This may result in a second dose, potentially resulting in acute toxicity. Recommended therapeutic doses are 250 to 750 mg/d, with a 250 mg dose achieving peak plasma concentrations averaging 3.4 µg/mL in healthy, young adults. Recreational users often consume 1 to 2 g/d, with upwards of 8 to 16 g/d. The male in our case presented a week prior to the Emergency Department indicating he was high and paranoid. He admitted use of clonazepam, marijuana and methamphetamine. A day before being found deceased, he was heard stomping around his apartment, which was described as uncharacteristic. He had previous suicidal ideation and a history of substance abuse. None of the other drugs detected in the screen had concentrations outside of therapeutic range. Cause of death is pending review by the Medical Examiner.

POSTER

P113

Stability of Ethanol in Electronic Cigarette Liquid Formulations

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Abstract

Introduction: Ethanol has been identified as a component of electronic cigarette (e-cig) liquids (e-liquids) in concentrations greater than 20%. Limited data regarding the health and safety implications of vaping ethanol exists. A recent clinical study found that ethanol was detected by a preliminary breath test instrument after participants vaped an ethanol-positive e-liquid. Ethanol is a controlled, pharmacologically active substance, and inhalation via vaping could lead to unknown effects and have significant implications. The long-term stability of ethanol in e-liquids is unknown.

Objectives:To demonstrate the stability of ethanol in e-liquids at different temperatures.

Methods: Seven hundred and fifty e-liquids were prepared by a commercial manufacturer (Kai's Virgin Vapors, Vista, CA) to contain 0%, 5%, or 20% ethanol in an e-liquid made of a 50:50 mixture of propylene glycol (PG) and vegetable glycerin (VG) in 5mL amber vials used by the e-liquid manufacturer. No nicotine, flavoring chemicals, or other ingredients were included in the formulation. Six e-liquids from each ethanol concentration were selected at random and sampled to determine baseline ethanol concentrations. The samples were stored in an oven (50 ℃), refrigerated (~5 ℃), frozen (-20 ℃), or at room temperature (~23 ℃), and sampled periodically. Ethanol concentrations were determined using a previously published method for headspace-gas chromatography-dual flame ionization detection (HS-GC-FID) and employed a Shimadzu HS-20 headspace sampler attached to a Nexis 2030 GC-dual FID. The method used matrix-matched (50:50 PG:VG) quality controls, had a linear range from 100-3000 mg/L, and an administratively set limit of quantitation of 100 mg/L.

Results: Average ethanol concentrations in the 0%, 5%, and 20% ethanol e-liquids stored at 23 °C across 1 year were determined to be 0.1% ± 0.02%, 5.6% ± 0.3%, and 20.0% ± 1.1%, respectively. In 5 °C conditions across 1 year, average ethanol concentrations were determined to be 0.1% ± 0.02%, 5.7% ± 0.3%, and 19.8% ± 1.3%, respectively. In -20 °C conditions across 2 years, average ethanol concentrations were determined to be 0.1% ± 0.03%, 5.4% ± 0.4%, and 20.7% ± 1.3%, respectively. In the 50 °C condition across 2 months, average ethanol concentrations were determined to be 0.1% ± 0.03%, 5.3% ± 0.5%, and 20.7 ± 0.7%, respectively.

Discussion: The results of this study demonstrated that across all three ethanol concentrations and storage conditions, ethanol content remained stable. Further, due to the sampling strategy, it was shown that neither the freeze-thaw cycles nor the increasing headspace in the 5 mL vials affected ethanol stability. Results describe the ethanol stability of an e-liquid stored in a glass vial, over a typical e-liquid life span and potential storage conditions. E-liquids purchased in plastic squeeze bottles or improperly closed may experience ethanol evaporation. E-cig users, substance use treatment counsellors, law enforcement officers, and other stakeholders should be aware of possibility of e-liquids containing ethanol.

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POSTER

P114

Determining Cannabinoid Acetate Analog (delta 9-THC-O-A, delta 8-THC-O-A, and CBD-di-O-A) Metabolites Using Rodent Brains

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Abstract

Introduction: The legality of various cannabinoids and their analogs is complex and varies between federal and state regulations. The Agricultural Improvement Act of 2018 (2018 Farm Bill) defined 'hemp', but this has only further clouded the legality of cannabinoids. Reports indicate that the acetate analogs: delta 9-Tetrahydrocannabinol-O-acetate (delta 9-THC-O-A), delta 8-Tetrahydrocannabinol-O-acetate (delta 8-THC-O-A), and cannabidiol di-O-acetate (CBD-di-O-A); are classified as Schedule I controlled substances as they do not occur naturally in cannabis plants and can only be obtained synthetically. However, these synthetic cannabinoids are commercially available in many products including electronic cigarettes and gummies. Currently, there is little research on the effects of the cannabinoid acetate analogs and limited information on their detection in biological matrices.

Delta 9-THC-O-A, delta 8-THC-O-A, and CBD-di-O-A are synthesized from different cannabinoids by an acetic anhydride reaction, similar to the synthesis of heroin from morphine. Each analog can be synthesized from delta 9-THC and delta 8-THC. CBD-di-O-A can also be synthesized from CBD. The acetate analogs are formed by the addition of an acetyl group to each hydroxyl group present on the original cannabinoid structures. Although there's little information known about the effects of delta 8-THC-O-A and CBD-di-O-A, antidotal reports indicate that delta 9-THC-O-A is a "spiritual cannabinoid" due to its psychedelic properties of producing vivid hallucinations.

These cannabinoid analogs have caused reason for concern due to their increasing prevalence in gummies and vapes. Currently, there is no presented research on how the cannabinoid acetate analogs are metabolized. Due to the structural similarity between the acetate analogs and the acetate analog of morphine, their initial metabolism is suspected to be similar to heroin.

Objectives: The objective is to determine the major metabolic metabolites of the cannabinoid analogs using brain homogenates and verifying their structure by utilizing liquid chromatography mass spectrometry/mass spectrometry (LC-MS/MS).

Methods: Homogenized rodent brains were used in five different test subjects: negative control, positive control (heroin), and three acetate analogs (delta 9-THC-O-A, delta 8-THC-O-A, and CBD-di-O-A) in triplicate.

The brain homogenate (200 µL) was pipetted into 132 separate tubes and processed at different time intervals: 2, 4, 8, 16, and 32 minutes. The negative control was only processed at 2 and 32 minutes as this was expected to have no reaction. The tubes were separated into two groups, 66 tubes were processed at 37°C (body temperature) and the other 66 tubes were processed at 24°C (room temperature). Different temperatures were utilized to observe normal formation of metabolites at body temperature and the effect a cooler temperature may have on metabolite formation.

Once pre-incubation was completed for all tubes, 20 µL of the tubes' respective analog (10 ng/µL) was added to start the reaction. Acetonitrile (100 µL) was added to the tube at the samples' respective time interval and placed immediately into the freezer to stop the reaction. The tube's contents were transferred into labeled vials for LC-MS/MS analysis.

Results: The brain metabolized the cannabinoid acetate analogs to their respective parent compounds. The expected metabolites were observed in the positive control. There were no metabolites observed in the negative control.

Discussion: The cannabinoid acetate analogs metabolized in a similar manner to heroin, the acetyl moiety is cleaved from the hydroxyl functional group(s) on the molecule. The analog's rate of metabolism appeared to be faster at normal body temperature, which may affect the ability to detect unique acetate metabolites in urine.

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POSTER

P115

Evaluating the Impact of Expiration and Additives on Blood Ethanol Concentration Stability in Vacutainer® Gray Stopper Tubes

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Abstract

Introduction: Blood specimens for toxicology casework involving Driving Under the Influence (DUIs) cases and traffic accidents are collected in Vacutainer® gray stopper tubes (GSTs) and routinely analyzed at the Alabama Department of Forensic Sciences (ADFS). GSTs contain sodium fluoride which serves as an anti-microbial and potassium oxalate which serves as an anti-coagulant. Additionally, the GSTs are under vacuum which allows for the blood to fill up the tube when being collected. Expiration dates for the tubes are associated only with the vacuum ability of the tube, not for the additives. Historically, Vacutainer® tubes manufactured by Becton Dickinson (BD) have been used at ADFS. Recently a new company, American Molecular (AM), began producing these GSTs. Over the years, there have been a number of recalls issued by BD including a recall in 2019 associated with a specific lot of tubes potentially lacking additives.

Objectives: This study evaluated the stability of blood alcohol concentrations (BACs) for the new AM GSTs, expired BD tubes, and unexpired BD tubes with and without preservatives.

Methods: An ethanol stock (1.00 g/100 mL) was made by dispensing 10.0 g of absolute ethanol into a beaker, diluting with DI water, and transferring with repeated washings to a 1 L volumetric flask. The solution was then diluted to 1 L with DI water. Whole, human blood free of additives was purchased from Innovative Research. The blood was first homogenized prior to making blood stock solutions. Blood stock solutions were made at 0.08 % and 0.15 % by pipetting 8 mL and 15 mL, respectively, of the ethanol stock solution into a volumetric flask and diluting to 100 mL with blood; or using other similar proportions. Using the stock solutions of blood, GSTs were filled with 8 mL of blood, immediately closed upon filling, and inverted approximately 5 times. Four sets of GSTs were used; unexpired BD tubes with additives, BD tubes with additives that expired in May 2021, BD tubes without additives, and AM tubes. Tubes were stored at 4 °C and blood ethanol concentrations were determined for each experimental set at days 0, 7, 14, 30, 60, and 90 using dual-column headspace gas chromatography with flame ionization detection (HS/GC/FID). Each time point consisted of three tubes at each concentration, analyzed in duplicate.

Results: Specimens for each experimental variable were analyzed to establish initial concentrations. Tubes were re-analyzed at each time point to show the BAC stability over time. There were no ethanol increases from any experimental set at 0.08 % and 0.15 %. Furthermore, ethanol losses were noted - e.g., average losses for each experimental set at 0.15 % at day 60 ranged from 0.003 % to 0.009 %. Ethanol was stable in BD tubes with and without additives. The use of expired tubes had no impact on ethanol stability and testing accuracy. BD and AM tubes performed similarly and were deemed fit for purpose.

Discussion: In court, questions regarding the stability of ethanol in the collection tubes are common. The scrutiny of BAC stability arises when there are issues pertaining to proper collection, storage, and expiration dates. This study highlighted the stability of BACs in various situations including tube expiration and lack of the additives sodium fluoride and potassium oxalate. Therefore, the 2019 recall from BD regarding the potential lack of additives likely has no analytical impact on the ethanol result. Additionally, this study demonstrated the suitability of alcohol analysis and the stability of ethanol in the new type of GST manufactured by AM. This study illustrated that AM tubes were fit for purpose and demonstrate similar performance when compared to BD tubes that have been historically used.

POSTER

P116

Translating a Standard Curve between Drugs as a Proof of Concept to Quantify a Drug and its Metabolite in a Single Sample

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Abstract

Introduction: Multipoint internal calibration (MPIC) is a method of quantification that utilizes isotopically labeled internal standards as internal calibrators. The area response is proportional to concentration and is consistent between standards of the same drug despite the different number of deuterium atoms. This proportionality constant can be eliminated by dividing by a standard of the same drug, leaving a ratio of calibrator concentration to standard concentration. Therefore, a calibration curve can be constructed from multiple calibrators made of various drugs as long as they each have an isotopologue standard that is at a consistent concentration so that they are in similar terms.

Objective: The goal of this method is to show that a calibration curve can be made up of two different drugs.

Methods: Utilizing an LC-MS, samples of whole sheep's blood underwent analysis to determine the concentration of both amphetamine and methamphetamine. Calibrators of amphetamine-d6 at 10 ng/mL, amphetamine-d11 at 100 ng/mL, methamphetamine-d9 at 600 ng/mL, and methamphetamine-d14 at 1000 ng/mL were used to construct a calibration curve with amphetamine isotopologues ratioed to the amphetamine-d8 at 500 ng/mL internal standard and methamphetamine isotopologues ratioed to the methamphetamine-d11 at 500 ng/mL. When quantifying, the intensity of the unknown undeuterated drug was ratioed to the analogous isotopically labeled internal standard. The Cuyahoga County Medical Examiner's Office's validated procedure for the quantification of amines was utilized in the sample extraction.

Results: A linear calibration curve was developed. Tests were performed to determine that the ratio of amphetamine to methamphetamine are equivalent in the same sample at the same concentration both pre- and post-extraction.

Discussion: This proof of concept has yielded promising results. Future work would pursue the quantification of metabolites utilizing a similar method. Combined calibration curves can be further developed and implemented .

POSTER

P117

A Statistical Comparison of Fentanyl and 4-ANPP in the Postmortem Population of Washington, DC

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Abstract

Introduction: Fentanyl and its analogs have been the driving force in behind the evolving opioid epidemic in recent years. In 2021, opioids were involved in 80,411 fatal overdoses in the United States. Synthetic opioids, including fentanyl, were responsible for 71,238 (88%) of opioid-involved fatal overdoses. Washington, DC has seen fatal opioid overdoses with similar trends to those of the nation. Deaths that involve an opioid have increased since 2018, reaching 459 opioid-involved deaths in 2022. Of these deaths, fentanyl was identified in 94% of cases. The percentage of heroin-involved deaths has steadily decreased since 2017 in DC's postmortem population.

Despropionyl Fentanyl (4-ANPP) is an inactive minor metabolite of fentanyl formed through amide hydrolysis. 4-ANPP is not excreted in the urine. 4-ANPP is also a precursor used in the "Seigfried" synthesis of fentanyl.

As of this writing, little information is available regarding the differentiation of 4-ANPP as a metabolite and/or as a precursor in postmortem samples. This information could be useful to medical examiners when determining cause and manner of death.

Objectives: The goal of this work is to determine the statistical significance of fentanyl and 4-ANPP levels in postmortem samples. This work will assess if the role of 4-ANPP as a precursor or a metabolite can be evaluated postmortem.

Methods: A previously validated LC-MS/MS method for the detection of fentanyl, 4-ANPP, and fentanyl analogs was used. An Agilent 1290 Infinity II liquid chromatograph coupled with a 6470 tandem mass spectrometer was used for analysis. Compounds were eluted using a mobile phase gradient consisting of 5 mM ammonium formate, pH 3.00 and 0.1% formic acid in acetonitrile. The total run time was 10 minutes. The method used was a qualitative, decision point assay. Therefore, peak abundances of fentanyl and 4-ANPP were used as opposed to concentrations. Statistics were performed with GraphPad Prism 9.

Results: A total of 878 cases were evaluated. Fentanyl and 4-ANPP had non-normal distributions of peak abundances. Thus, non-parametric statistical analyses were performed. A Wilcoxon test was performed and yielded a p value < 0.0001. Correlation of the data was accessed with non-parametric Spearman coefficient, which was 0.7415.

Discussion: The Wilcoxon test is used to determine the statistical significance of sets of pairs. The pairs of fentanyl and 4-ANPP ratios were found to be statistically significant at the 95% confidence level. The correlation coefficient of 0.54 shows that 54% of the variation between fentanyl and 4-ANPP is explained. While the data is statistically significant, the correlation is relatively poor. Thus, the role of 4-ANPP as a precursor versus as a metabolite could not be determined in our data set. The addition of more variables, such as the presence of other fentanyl precursors and metabolites, could improve the correlation between fentanyl and 4-ANPP in postmortem samples.

POSTER

P118

Miniaturization Strategies for Streamlined Drugs of Abuse Extraction prior to UHPLC-MS/MS Analysis

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Abstract

Introduction: Miniaturized sample preparation, particularly solid phase extraction (SPE), has gained popularity in recent years with perceived benefits such as lower solvent consumption, sample processing and turnaround time improvements. However, implementation rarely results in total workflow solution with multiple pain points still evident. One of the greatest workflow compatibility issues is related to the SPE elution solvents and LC/MS mobile phases. This incompatibility often results in continued necessity of an evaporation step. While elution solvent volumes are much lower this can still be somewhat of a bottleneck. Here we investigate alternative solid phase extraction chemistries to provide a complete workflow solution resulting in elimination of the evaporation step prior to LC-MS/MS analysis.

Objectives: This poster will present strategies for automating drugs of abuse extraction, eliminating evaporation steps while maintaining sensitivity using a dedicated low volume sample preparation format.

Methods: A typical drugs of abuse target analyte panel was spiked at various concentrations and extracted from non-hydrolysed and enzymatically hydrolysed human urine. Sample extractions were investigated using polymer-based solid phase extraction: comparing traditional reversed-phase and mixed-mode weak cation exchange chemistries. For increased sensitivity assay miniaturisation was performed using the Biotage® Mikro 2 mg (low volume plate) SPE format. Final low volume extraction protocols were transferred to the Extrahera LV-200 automated sample preparation workstation. UHPLC-MS/MS analysis was performed using Nexera UHPLC coupled to an 8060 triple quadrupole MS system. Chromatography utilised traditional mobile phases of ammonium formate and formic acid in water and MeOH and separation afforded by the Raptor Biphenyl analytical column.

Results: A drugs of abuse panel including amphetamines, opiates, benzodiazepines, cocaine and other regularly analysed drugs were extracted from urine using polymer-based reversed phase and the corresponding mixed-mode weak cation exchange SPE chemistries in 10 mg 96-well plate format. Strong cation exchange SPE chemistry was ruled out due to necessity of high pH elution conditions which were incompatible with LC mobile phases. Initial evaluations of the polymeric reversed phase chemistry delivered reproducible analyte recoveries, typically greater than 80% depending on drug polarity and wash solvent composition. Further investigation with respect to matrix factors demonstrated very different results. High matrix effects were observed on many mid to non-polar analytes such as the benzodiazepines. Wash solvent, pH and elution solvent optimisation did not adequately remove the matrix effects ultimately ruling out this chemistry option. Weak cation exchange SPE optimisation delivered recoveries great than 80% for all but a few target analytes such as benzoylecgonine and the 7-amino-benzodiazepine metabolites. Matrix factors were substantially better compared to the corresponding reversed-phase chemistry data. Miniaturisation to the Biotage® Mikro 2 mg WCX (low volume plate) format demonstrated excellent scalability. Further optimisation was performed for all steps in terms of pH control, ionic strength and volumes. Final elution volumes of 30 µL were achieved using the optimised method. Comparison of direct injection of the elution solvent to pre-dilution prior to injection for chromatographic peak shapes and injector reproducibility demonstrated the latter approach to be our preferred option. Ultimately, either approach could be used order to allow the elimination of evaporation steps. The extraction protocol was transferred and optimised using Extrahera™ LV-200 automated sample preparation platform, demonstrating excellent correlation to manual PPM-96 processing. Final method performance and calibration curves demonstrated excellent linearity and coefficients of determination, r2 > 0.99 for all analytes while delivering sub ng/mL LOQs.

Conclusion: WCX chemistry provided a better approach to drugs of abuse testing while allowing the elimination of evaporation steps for improved workflow. Good correlation was observed scaling chemistry from 10 mg to 2 mg plate options and also between manual and automated processing of the final method.

POSTER

P119

Harmonizing Out the Variability of an LC-MS/MS Instrument Fleet

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Abstract

Introduction: Liquid chromatography-tandem mass spectrometry (LC-MS/MS) plays a vital role in Forensic Toxicology. However, a lack of standardized performance across instruments poses many challenges. Vendor performance specifications are not adequate indicators of true instrument performance in production. Instruments of the same model and configuration can often possess inherent personality differences impacting reproducibility, sensitivity, and ultimately adding biases into test results. To harmonize data generated on multiple systems, a benchmark process was developed and deployed to identify problematic systems, diagnose the cause of poor performance, and normalize instrument performance for improved analytical consistency.

Objective: Develop and implement a standardized process to diagnose key factors influencing LC-MS/MS performance to achieve robust and reproducible analytical outcomes.

Method: A benchmark LC-MS/MS method was developed employing a mixture of 17 performance indicating compounds comprising of benzodiazepines, opioids, stimulants and cannabinoids. The chosen compounds include a variety of small molecules relevant to toxicology testing which are utilized to evaluate specific aspects of the LC-MS/MS system impacting instrument performance. Within the test mixture, selected compounds highlight performance differences in the following areas: ionization efficiency, collision induced fragmentation, cleanliness and calibration of the quadrupoles, mobile phase quality, autosampler delivery, and chromatographic variability. Both negative and positive electrospray ionization are evaluated. Systems are tested at least every six weeks and when routine performance indicates a potential problem. Results are compared to both the historic performance of the specific instrument, and the current performance of instruments of the same model and configuration.

Results/Discussion: One-hundred and twenty LC-MS/MS systems (SciexTM MS coupled with WatersTM Acquity UPLC) comprised of six primary model configurations and located at four unique geographical locations were monitored using the above-described procedure. Data compiled over multiple years provided the baseline used to achieve system harmonization. Numerous scenarios have been encountered where systems met the manufacturer's performance specifications, yet critical performance differences were identified through the deployment of the benchmarking procedure. As benchmarking data are collected on a system, they are compared to both the system's own historic performance, and the performance of similar systems. Performance discrepancies impacting individual compounds are semi-diagnostic, allowing many root causes to be quickly identified and addressed. Only then can the system in question be returned to the standardized fleet of instruments.

The diagnostic mix is utilized to harmonize chromatographic as well as MS performance. Retention time deviations greater than 6 seconds have been successfully used to diagnose leaks and LC pumping flaws impacting individual channels. Similarly, chromatographic aberrations with the first eluting compound in the mix (morphine) have been utilized to identify errors in the injector. Other select compounds, such as D8-amphetamine, were utilized to identify MS tuning and calibration discrepancies. A loss of sensitivity only impacting the benzodiazepines within the mixture indicates improperly prepared or expired mobile phase, while a loss of benzoylecgonine response is diagnostic of ionization issues. Source heating issues can be identified by aberrations in the signal for amphetamine, and sensitivity changes differentially impacting negative and positive ionization compounds implicates dirty quadrupoles.

Systems identified as being overly sensitive are intentionally detuned to ensure the upper limits of the assay dynamic range is not compromised. Intentional detuning can be achieved by targeting the source, detector, quadrupole, or autosampler settings.

By adopting a diagnostic harmonization strategy, laboratories can produce consistent and reproducible results across an entire fleet of instruments. Underperforming instruments can quickly be identified while simultaneously obtaining diagnostic information about the cause of performance gaps.

POSTER

P120

Comprehensive novel psychoactive substance (NPS) and synthetic opioids screening in dried blood spots (DBS) using HRMS

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Abstract

Introduction: The prevalence of novel psychoactive substances (NPS) on the recreational drug market continues to fuel the ongoing opioid crisis. The emergence of new substances has been linked to a drastic increase in intoxications and accidental fatalities which pose serious public health risks and legal challenges. NPS screening can be performed in a variety of biological matrices such as blood, serum, plasma or urine. In recent years, the use of dried blood spots (DBS) has become a straightforward alternative that significantly simplifies and shortens sample preparation compared to other approaches.

Objectives: In this study, a comprehensive workflow is presented that uses the SCIEX X500R QTOF system in combination with a fast and optimized DBS sample extraction procedure for the detection of a panel of 132 NPS including synthetic cannabinoids, synthetic cathinones, dissociatives, hallucinogens, fentanyl analogs, synthetic opioids and some metabolites. This robust NPS screening workflow provides reproducible, accurate and precise quantification of low level of analytes with a wide range of physical and chemical properties.

Methods: A total of 132 molecules and metabolites including 53 synthetic cannabinoids, 47 synthetic cathinones, dissociative and hallucinogens, 32 fentanyl analogs and synthetic opioids were studied. Protein saver cards (also known as DBS cards) were purchased from Whatman. Human whole blood calibrator samples spiked with various concentrations of the 132 molecules were spotted onto the DBS cards. NPS were extracted using a simple extraction procedure. UHPLC separation was performed using the SCIEX ExionLC AC system and a Phenomenex C18 column. Mobile phases used consisted of water, acetonitrile and modifiers. MS and MS/MS data were collected for each sample using SWATH acquisition on the SCIEX X500R QTOF system in positive ionization mode. Data acquisition was TOF MS scan followed by 18 MS/MS scans using variably sized Q1 windows covering a mass range from 100 to 575 Da. Data was acquired using SCIEX OS software 1.5.

Results and Discussion: The sample extraction efficiency was investigated as well as the ion suppression and enhancement for the 132 molecules included in this panel. The recovery values ranged between 31 and 98% (average of 54%) and the matrix effects compensated by internal standard ranged between -69% and 10% (average of -28%), proving the efficacy of the sample extraction method used in this study.

Reliable quantification of drugs extracted from DBS is critical to ensure the robustness of a developed method. In this study, SWATH acquisition was used as the detection method to generate comprehensive and high-quality MS/MS spectra of every detectable NPS extracted from the DBS cards. The series of five calibrator solutions ranging from 5 to 100 ng/mL were injected to evaluate the quantitative performance of SWATH acquisition and its ability to accurately measure various levels of NPS extracted from DBS with a high level of linearity, precision and accuracy. Limits of quantification (LOQs) ranged from 6 ng/mL for 1-(5-fluoropentyl)-N-(2-phenylpropan-2-yl)-1H-indazole-3-carboxamide (5F-CUMYL PINACA) and 3,4-dicloro-N-[(1-(dimetilammino)cicloesil)metil] benzamide (AH-7921) up to 15 ng/mL for 2-(2,5-dimethoxy-4-propylphenyl)ethan-1-amine (2C-B). A series of injections were performed over the course of two consecutive days only to evaluate the reproducibility and robustness of the method. Two days was considered to be representative of a large analysis batch encountered by most labs. Calculated concentration accuracies (%CV) were found to be within 80% and 120% for most NPS, precision (%bias) below 30% for synthetic cannabinoids and below 20% for all other NPS classes. Overall, the assay showed great reproducibility over the course of two consecutive days and across the five calibrator solutions, proving the quantitative robustness of the overall workflow.

POSTER

P121

NPS Surveillance through High Resolution Mass Spectrometry Screening of Forensic Samples: Insights and 2022 Trends

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Abstract

High resolution mass spectrometry (HRMS) instruments such as liquid chromatography/time-of-flight mass spectrometry (LC-TOF/MS) can collect untargeted accurate mass data which permits identification of known and unknown compounds in a single analytical run. Forensic laboratories are leveraging non-targeted acquisition capabilities as a tool for the identification of novel psychoactive substances (NPS). Mining HRMS data, either retrospectively or through a supplemental surveillance library, allows for identification of new substances without re-analysis or costly time delays for updating analytical scopes. This is critical in efforts to rapidly report NPS, since these substances continually emerge and often have short lifecycles. However, supplemental compound information from data-mining is subject to limitations and requires careful scrutiny. LC-TOF/MS supplemental findings should be evaluated so that further testing is not pursued on false positives from interferences or decomposition products.

This presentation reviews the use of a surveillance library in mining HRMS data collected during the course of comprehensive screening of predominantly blood and urine samples collected during forensic investigations, predominantly for the detection of NPS, and 2022 trend information gleaned from compiling the results.

A supplemental surveillance library covering a curated scope of NPS (excluding synthetic cannabinoid receptor agonists) is employed alongside targeted reporting, for authentic forensic samples that undergo comprehensive postmortem or impaired driving screening. The in-house library is built by analyzing standard reference materials through established protocols. Compounds listed in the surveillance library are not verified against daily calibration or control material, allowing for rapid and flexible updates. Positive matches are evaluated for retention time shift, chromatography, area response, and other supportive indicators. If a positive match meets established criteria, confirmatory testing is recommended and submitted agency must approve additional testing.

Data linked to the surveillance library findings in 2022, including analyte(s) and additional testing performed, were compiled to evaluate the effectiveness of the process as well as identify emerging NPS trends. In the 2022 calendar year, there were 3003 out of scope findings detected in 2413 specimens which were screened using LC-TOF/MS. Confirmatory testing was recommended in 2379 instances (79%). Further testing was approved in 1528 cases with a final confirmation rate of 92%.

Bromazolam accounted for the majority of additional NPS findings with 1147 detections in samples screened in 2022. However, further testing was only recommended in 911 cases (79%). Additional testing was approved in 615 cases and bromazolam was confirmed in 584 samples, for an approximate 95% confirmation rate. Other designer benzodiazepines commonly detected and reported included clonazolam's metabolite, 8-aminoclonazolam (n=128), flualprazolam (n=116), and deschloroetizolam (n=17).

The emerging stimulant N,N-dimethylpentylone was added to the scope of the surveillance library in April 2022, after a rise of pentylone detections prompted further investigation. N,N-Dimethylpentylone and its metabolite pentylone were the most frequently detected synthetic stimulants in 2022, but were confirmed 31 and 67 times, respectively. Eutylone was also confirmed in 18 cases.

Novel synthetic opioids (NSOs) continue to be a dynamic subclass of NPS. The fentanyl analogue para-fluorofentanyl continues to be detected, almost exclusively in conjunction with fentanyl. Other NSOs included several analogues of the nitazene subclass: isotonitazene (n=28), protonitazene (n=26), metonitazene (n=40), N-pyrrolidino etonitazene (n=3), and butonitazene (n=1). Additionally, brorphine (n=20) and 2-methyl AP-237 (n=2) were also detected. Tianeptine, an antidepressant used off-label for atypical opioid agonist activity, was confirmed in 29 cases.

POSTER

The implementation of concurrent processing of HRMS data with targeted scopes and a surveillance library has allowed for a more rapid response in identifying and reporting emerging substances in forensic toxicology casework. When thoughtfully implemented and evaluated, supplemental compound information can reveal critical findings, including NPS, which can be aggregated for trend analysis.

P122

Prevalence of Ketamine in Hair and Nails in High-Risk Populations

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Abstract

Introduction: Ketamine is a powerful anesthetic drug that has been used for both veterinary and human purposes. It has become increasingly popular for its recreational use in recent years. Owing to its widespread use, developing reliable methods for detecting its presence in biological samples has become important. Keratinized specimens like hair and nail are particularly useful matrices for drug testing, as they provide a longer detection window of drug use. The potential applications of ketamine testing in hair and nails include use in forensic investigations and drug treatment programs. USDTL developed a confirmation method for ketamine in keratinized specimens using LC-MS/MS.

Objectives: This study aims to determine the prevalence and concentration of ketamine and norketamine in human hair and nail samples in a high-risk population using LC-MS/MS analysis.

Methods: A total of 6131 samples previously tested for ketamine during the study period were evaluated. After initial screening, presumptive positives were subjected to confirmation testing. Roughly 20 mg of hair or nails were washed with acetone, dried, and pulverized. Isotopic internal standards and 3mL of 0.1 M HCl were added and the mixture was incubated at 53°C for 2 hours before being subjected to solid phase extraction using a mixed-mode, cation exchange column, followed by LC-MS/MS analysis. Both ketamine and norketamine were analyzed, with a cutoff of 100 pg/mg.

Results: The 6131 keratinized specimens consisted of 866 nails and 5267 hair samples. Ketamine and norketamine were both positive in 68 hair specimens, with 46 positives for ketamine only. In nails, 5 confirmed positive for both ketamine and norketamine, while 6 confirmed positive for ketamine only. Of all positive hair samples, 92 were head hair, 12 were body hair, and 10 were unidentified. Positive nails consisted of 8 fingernails, 1 toenail, and 1 unidentified. Positivity rates were 2.16% and 1.27% for hair and nail, respectively, with an overall positivity rate of 2.04%. In hair, the median concentrations for ketamine and norketamine were 543 pg/mg (Range=102 –171747pg/mg), and 566 pg/mg (Range=103 – 31211 pg/mg), respectively. In nails, the median concentrations for ketamine and norketamine were 1437 pg/mg and (Range=175 – 419511 pg/mg), and 1377 pg/mg (Range= 208 – 13896 pg/mg), respectively.

Discussion: Hair positivity rates were higher compared to nails, making it the preferred keratinized matrix. Conversely, the higher median concentration in nails highlights their usefulness in cases where longer detection windows are desired. The results of this study offer a significant understanding of the frequency and dispersion of ketamine and norketamine in the keratinized specimens examined. Further research is needed to fully understand the implications of these results for clinical and forensic applications.

POSTER

P123

Prevalence of Fluorofentanyl and Designer Opioids in Alabama 2021-2023

Karen Valencia, Kahleah Pell, Clarissa Lawen, Curt Harper

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Abstract

Introduction: Fentanyl is a synthetic opioid which has been used as an adjunct to anesthesia and as a narcotic analgesic, particularly for patients suffering from cancer, and it is estimated to be up to 100 times stronger than morphine and up to 50 times stronger than heroin. Its ease of manufacture and high potency make it an attractive alternative to heroin thus contributing to the opioid epidemic in the United States. In order to subvert laws specific to the traditional opioids, analogues of fentanyl and designer opioids have been produced and distributed. Fluorofentanyl is the most recent fentanyl analogue that is being seen in labs across the country and is usually combined with fentanyl. The Alabama Department of Forensic Sciences (ADFS) analyzes samples in both human performance and death cases and has been tracking fentanyl analogues and designer opioids since the fentanyl analogues appeared in casework in 2015.

Objectives: To illustrate the prevalence of fluorofentanyl and other designer opioids detected in toxicology samples reported by ADFS between January 1st 2021 and June 1st 2023.

Methods: Blood samples were screened for fentanyl by enzyme immunoassay using either the Randox Evidence Plus Analyzer utilizing the DOA Ultra WB reagents or the Tecan Freedom Evo75 with kits from Immunanalysis and confirmed with a liquid-liquid extraction followed by liquid chromatography/tandem mass spectrometry (LC/MS/MS) analysis using an Agilent 6460 or 6470 instrument. Fluorofentanyl was established to be cross reactive with the DOA Ultra WB reagents by in-house analysis. The confirmatory analysis contained designer opioids as well as the fentanyl/fentanyl analogues and positive results for the designer opioids were confirmed with a repeat analysis. Cases with suspected opioid use which did not screen positive on the immunoassay were also analyzed by this method. A drug search for fluorofentanyl and designer opioids was performed via the Laboratory Information Management System (LIMS) for toxicology cases from January 1st 2021-June 1st 2023. Two case studies were highlighted to demonstrate the similarities in the presence of flourofentanyl and designer opioids between autopsy and traffic case types.

Results: Between January 2021-January 2022 cases reported containing fluorofentanyl increased 410%. In the first half of 2023, fluorofentanyl decreased 40% compared to the second half of 2022. Cases reported to contain carfentanil or brorphine significantly decreased while the nitazines increased 300% from 2021-2022 and show an increase of 200% from the second half of 2022 through the first half of 2023.

Discussion: Fluorofentanyl and designer opioids, particularly the nitazine class, continued to be an issue in Alabama with an increase in the number of cases containing these substances reported each year. The Alabama Department of Forensic Sciences continues to evaluate fluorofentanyl and designer opioid prevalence to guide method development. Quarterly reports from the Center for Forensic Science Research and Education as well as reports from the Drug Chemistry section at ADFS are utilized to monitor which new compounds may need to be added to our scope of analysis. As the opioid epidemic in the United States continues, laboratories across the country will need to be able to identify the newer compounds, thus providing law enforcement and medical examiners appropriate data for case management.

POSTER

P124

Automated Detection and Re-Analysis of Over-Range and Saturating Samples

Matthew Campbell[1], William Slade[1], Brian Rappold[1], Gregory Janis[2]

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Abstract

Introduction: LC-MS analysis of samples with exorbitantly high concentrations of analyte can result in poor analytical performance. High analyte concentrations can overload the column or saturate the MS detector, resulting in any number of undesirable features including poor peak shape, altered retention times, failed transition ratios, suppression of isotopic internal standards, and inaccurate quantitation. Typically, these samples must be manually identified, diluted, and then re-analyzed. Such a strategy essentially doubles the time required for analysis. Analyte overload can be particularly troublesome for assays requiring low detection limits, but samples are observed at concentrations many orders of magnitude higher. Such is the case for testing of fentanyl and norfentanyl in human urine where concentrations can exceed 7,500 ng/mL. Herein, an automated process is described for detecting and responding to samples containing fentanyl and/or norfentanyl concentrations greater than the upper limit of quantitation.

Objectives: This work aims to accurately quantify and gauge quantitative and qualitative criteria when overloaded samples are detected in LC-MS/MS without pre- or post-extraction dilution. Saturated peaks are detected based on the peak height of the analytes, and then automatically rescheduled for re-injection using smaller injection volumes. Through reducing the amount of analyte injected into the system, peaks should return to having proper chromatography characteristics, including retention time, peak shape, transition ratios, and quantitation.

Methods: Urine samples are analyzed for fentanyl and norfentanyl through a simple 40X dilution/clarification procedure utilizing matched isotopic internal standards. Instrumental analysis occurs on a Waters Acquity LC coupled to a Sciex 5500 Triple Quadrupole MS, and covers a range of 0.475 - 50 ng/mL fentanyl and 1.9 - 200 ng/mL norfentanyl. The initial injection volume is set to 8 µL. Data processing utilizes Indigo Ascent, capable of "flagging" undesired peak characteristics. A sample is considered to be overloaded when quantitation exceeds the ULOQ, or the peak height is greater than 1 x 106 and one or more of the following "flags" are observed in Ascent: failed mass ratio, inconsistent retention time, or low peak fit quality. Samples with the described criteria are automatically queued for re-injection with an internally developed program interfacing with Sciex Analyst. Re-injections are performed with an injection volume of either 0.8 µL (10x dilution equivalent) or 0.2 µL (40x dilution equivalent), depending on level of saturation observed in the first injection. Peak height is used instead of peak area because overloaded peak shapes can cause erroneous area measurements.

Results: In our assay, the first signs of saturation occur around 1,000 ng/mL fentanyl or 2,500 ng/mL norfentanyl. Detector saturation is initially observed in the stronger, quantitative transition, and results in MRM ratio failures. Further increases in concentration exacerbates detector saturation and eventually yields unexpected peak shapes caused by chromatographic overload. Trigger points and the analytical accuracy of the technique was validated against a sample set containing high analyte concentrations and compared against the results using a traditional paradigm of dilution prior to re-analysis. Qualitative criteria initially impacted by the high analyte concentrations (peak shape, ratios, etc) consistently met acceptance following the automated re-injection protocol. Accurate quantitation is achieved despite the inherent lower recovery of the internal standards when using small injection volumes. IS recovery is graded against the initial injection.

Discussion: The low limit of quantitation necessary for detecting fentanyl in urine samples makes the assay particularly prone to overloading/saturation at the high concentrations frequently encountered. Automated small volume re-injection circumvents the need to identify, dilute, and re-analyze these samples. This method has or is being applied to other analytes, including but not limited to urine buprenorphine and multiple oral fluid assays.

POSTER

P125

The Difference a Glucuronide Can Make: Comparing the Stability of Psilocin vs Psilocin-glucuronide

Gregory Janis, Melissa Goggin

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Abstract

Background / Introduction: Many urine toxicology assays target free, unconjugated drugs despite the predominant excretion of glucuronide conjugated metabolites. Hydrolysis procedures are frequently chosen over directly measuring conjugated targets for multiple reasons, including a lack of commercially available reference materials for conjugated forms of the drugs of interest. When conjugated reference materials are unavailable, stability studies are frequently performed using unconjugated reference materials. Due to differences in solubility, adhesion, and chemical stability, sample stability information collected using unconjugated drugs may be irrelevant when attempting to predict the stability of conjugated drug forms.

The hallucinogenic compound psilocin is notoriously unstable. Psilocin is primarily excreted in urine as psilocin-glucuronide; however, the glucuronide conjugate is not commercially available. Fortified stability studies investigating free psilocin in urine indicate a rapid decay of the analyte unless samples are frozen and protected from light. Those studies may or may not be relevant to authentic samples.

Objectives: To evaluate the stability of both free and glucuronide conjugated psilocin, providing insight into how glucuronidation impacts psilocin stability in authentic urine samples.

Methods: Due to limited availability of reference material for glucuronide conjugated compounds, known positive samples were utilized to compare stability of free and glucuronide-conjugated psilocin. Psilocin levels were quantified using a validated LC-MS/MS method utilizing psilocin-d10 internal standard. Initial analyte concentrations were determined in aliquots analyzed with and without hydrolysis to stepwise measure free and total psilocin. The level of psilocin-glucuronide was equal to total psilocin measured in the hydrolyzed aliquot minus the free psilocin measured in the non-hydrolyzed aliquot. Following the initial determination, aliquots of the samples were stored frozen and at room temperature. Drug-free urine fortified with psilocin reference material was simultaneously evaluated for stability. Aliquots at the various storage conditions were evaluated with and without hydrolysis following 3, 7, and 14 days of storage.

Results/Discussion: Free psilocin was confirmed to be unstable at any of the room temperature conditions tested, while psilocin-glucuronide in positive samples was stable for up to 2 weeks at room temperature.

Fortified stability studies using free psilocin suggested samples should be shipped/stored frozen. However, in authentic samples containing psilocin-glucuronide, stability experiments indicate that ambient shipping/storage is sufficient.

Conclusion: This study shows the importance of testing assay limits based on the actual compound that is present in samples. Even though unconjugated drugs are targeted for quantitation, the glucuronide conjugated metabolites are generally more prevalent in urine samples and may be more stable than the free analyte.

POSTER

P126

Evaluating the efficacy of commercial oral fluid collection devices

Ashley Gesseck-Harris, Justin Poklis, Michelle Peace

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Abstract

Introduction: In 1988, HHS published the Mandatory Guidelines for Federal Agency Employee Drug Testing Programs in which initial and confirmatory cutoff levels for urine were set at concentrations used to demonstrate specific use of prohibited drugs. Despite being aware of the potential for cheating on a urine test, the department has approached collection of urine with a concern for employee privacy, only directly observing collections under specific circumstances to protect Fourth Amendment rights. In 2004, HHS solicited public comment on alternative testing that could be directly observed, including oral fluid, hair, and sweat. The addition of matrices that can be collected with direct observation could aid in combating the adulteration, substitution, and dilution of samples. In 2019, HHS deemed the science supporting oral fluid testing was appropriate for Federal employee testing with the same level of confidence that has been applied to urine.

Per Federal Register Vol. 87, No 67 from April 2022 oral fluid collection devices must meet the following minimum criteria: be FDA-cleared, contain a volume adequacy indicator, collect a minimum of 1 mL within ± 10% (if a diluent or other volume modifier is present), collection tube is sealable, non-leaking, and transparent buffer is within ± 2.5% of target volume, if applicable. Additionally, the recovery of drugs from the collection pad must be within 80 – 120% at (or near) the initial cut-off, and drugs must be stable (≥80% of concentration at time of collection) for 5 days at 18 – 25 °C. Per NLCP Oral Fluid Manual rev0222, the laboratory must validate that the collection device meets these requirements on a minimum of 3 different lots and at least 15 devices and 95% of devices should fall within the range.

Objectives: To evaluate the physical aspects of four different commercial oral fluid collection devices, including one buffer-less device, according to the HHS regulations and potential deviations from the requirements.

Methods: Oral fluid samples were collected from six individuals using four commercially available devices. The volume of oral fluid collected was determined by weighing the swab before and after collection. The volume of diluent was determined by weighing the liquid within the collection device and calculating by subtracting the weight of an empty, dry tube from the weight of the tube containing a buffer. All volumes were calculated both with an assumption of 1.000 g/mL density and normalized to the weight of 1 mL oral fluid or buffer from each donor or device.

Results: All devices failed at least one of the requirements set forth by HHS for oral fluid collection devices. The buffer-less collection device is not required to meet the 1 mL ± 10% requirement, but is expected to reliably collect 1 mL. This device had the highest standard deviation for all devices tested. The three buffered collection devices all fail either the volume collected or amount of buffer requirement. A limitation of this study was that only one lot was used for evaluation.

Discussion: With variable amounts of oral fluid collected and buffer, the value of the cutoff gets diminished, and both volumes can have impacts on the final outcome of testing. Even for volumes within the set requirement, whole saliva concentrations near the cutoff can change reporting category.

To obtain a more accurate result, a component to the buffer could be added to be analyzed by the laboratory. This component would be used to normalize the total volume of oral fluid and buffer for each collection. Alternatively, a collection device with no buffer may improve consistency of concentration between collections.

This project was supported in part by the National Institute of Health Grant (P30DA033934)

POSTER

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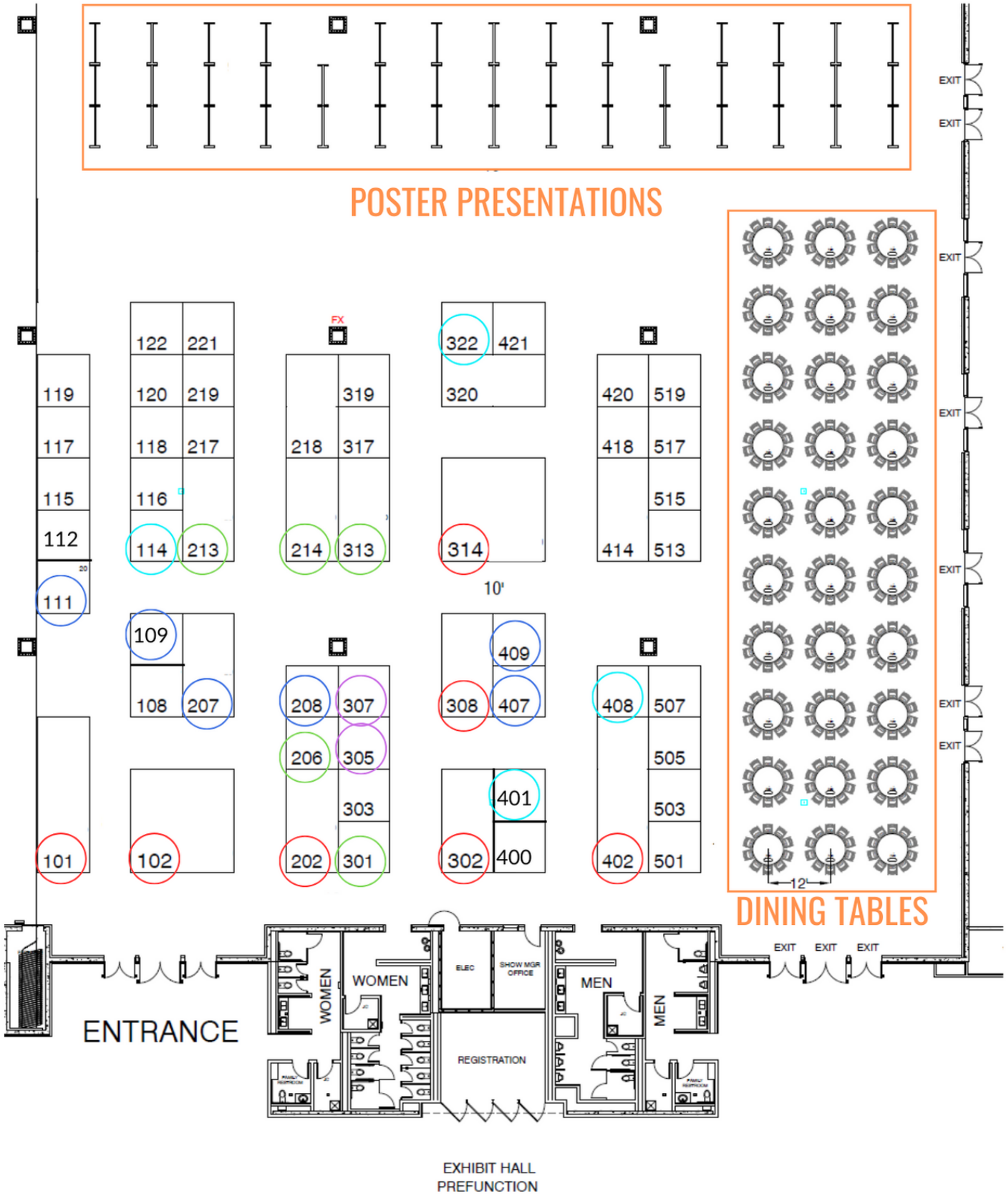

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Sharing knowledge is a core pillar of our work at the FTCOE. We actively promote the exchange of information, insights, and expertise through various channels, including workshops, webinars, podcasts, and publications. By fostering an environment of evidence to action, continuous learning, and professional development, we empower forensic scientists and practitioners to stay abreast of the latest research findings and emerging trends.

Recognizing that every forensic analysis presents unique challenges, we are committed to providing practical solutions. Our team of experts collaborates closely with stakeholders from the forensic community to identify and address the most critical issues. By supporting research, developing toolkits, and offering technology transition resources, we strive to equip practitioners with the necessary resources and guidance to overcome obstacles and achieve successful outcomes.

At the FTCOE, we value collaboration, integrity, and excellence. We believe that by bringing together diverse perspectives, sharing knowledge, and fostering innovation, we can drive meaningful progress in the field of forensic science. Whether you are a researcher, practitioner, or industry professional, we invite you to join us in our mission to advance technology, share knowledge, and provide solutions to the challenges that shape the future of forensic science. We strive to be an invaluable resource for all members of the diverse forensic science and criminal justice communities, evolving with the changing needs of the community and improving each day.

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JusticeTrax - Booth #: 501

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Kura Biotech FINDEN - Booth #:409

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MilliporeSigma - Booth #: 213

Tihana Tomas

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NMS Labs - Booth #: 118

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OraSure Technologies - Booth #: 505

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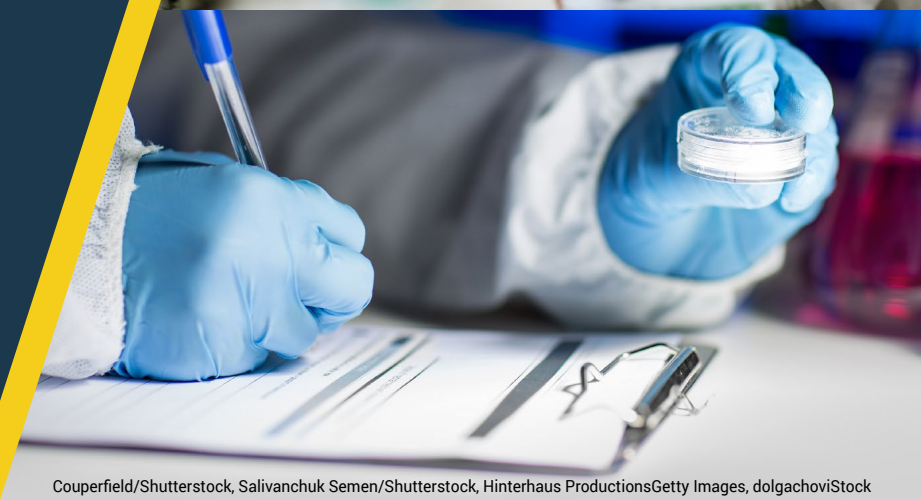

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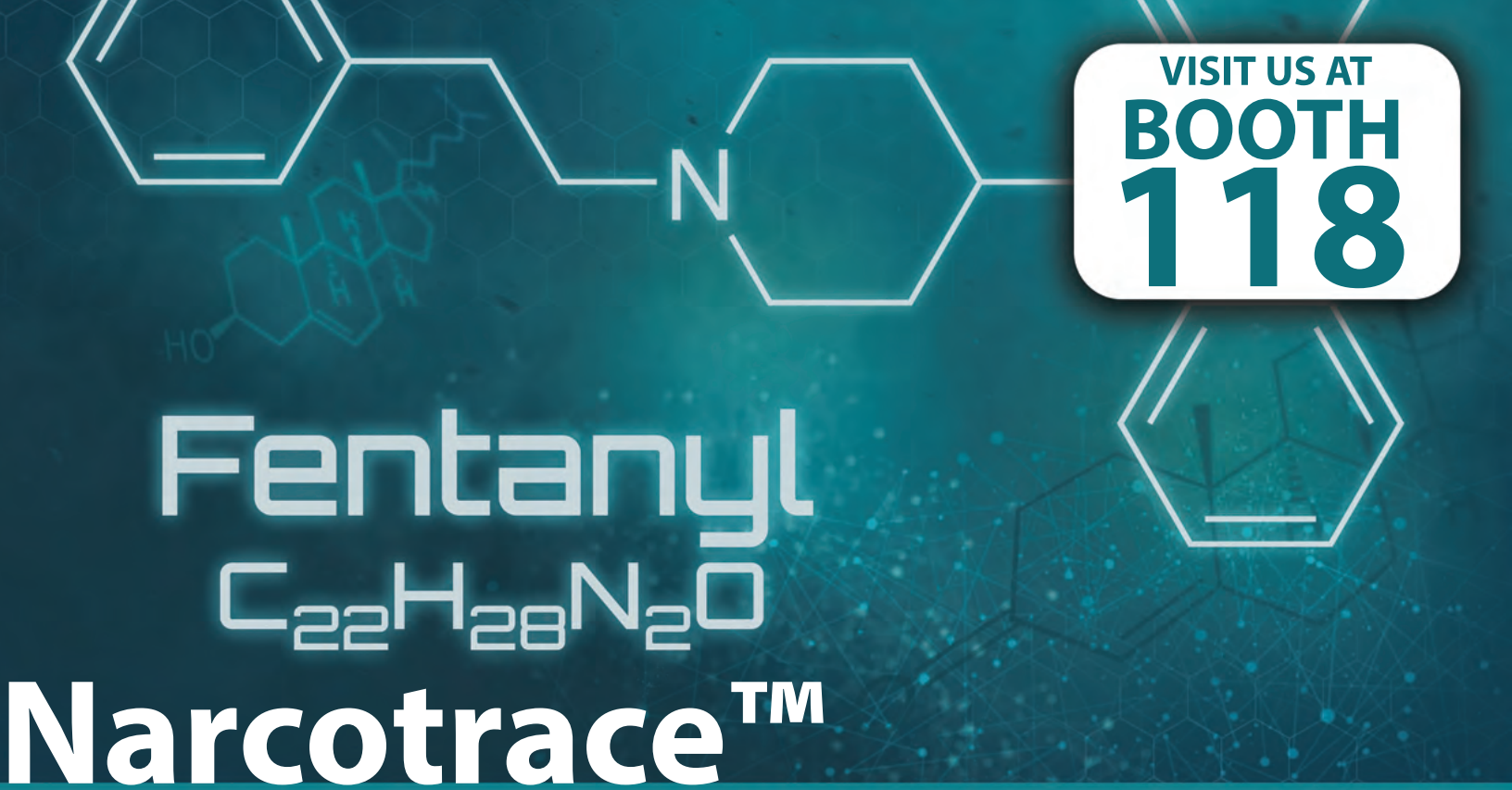

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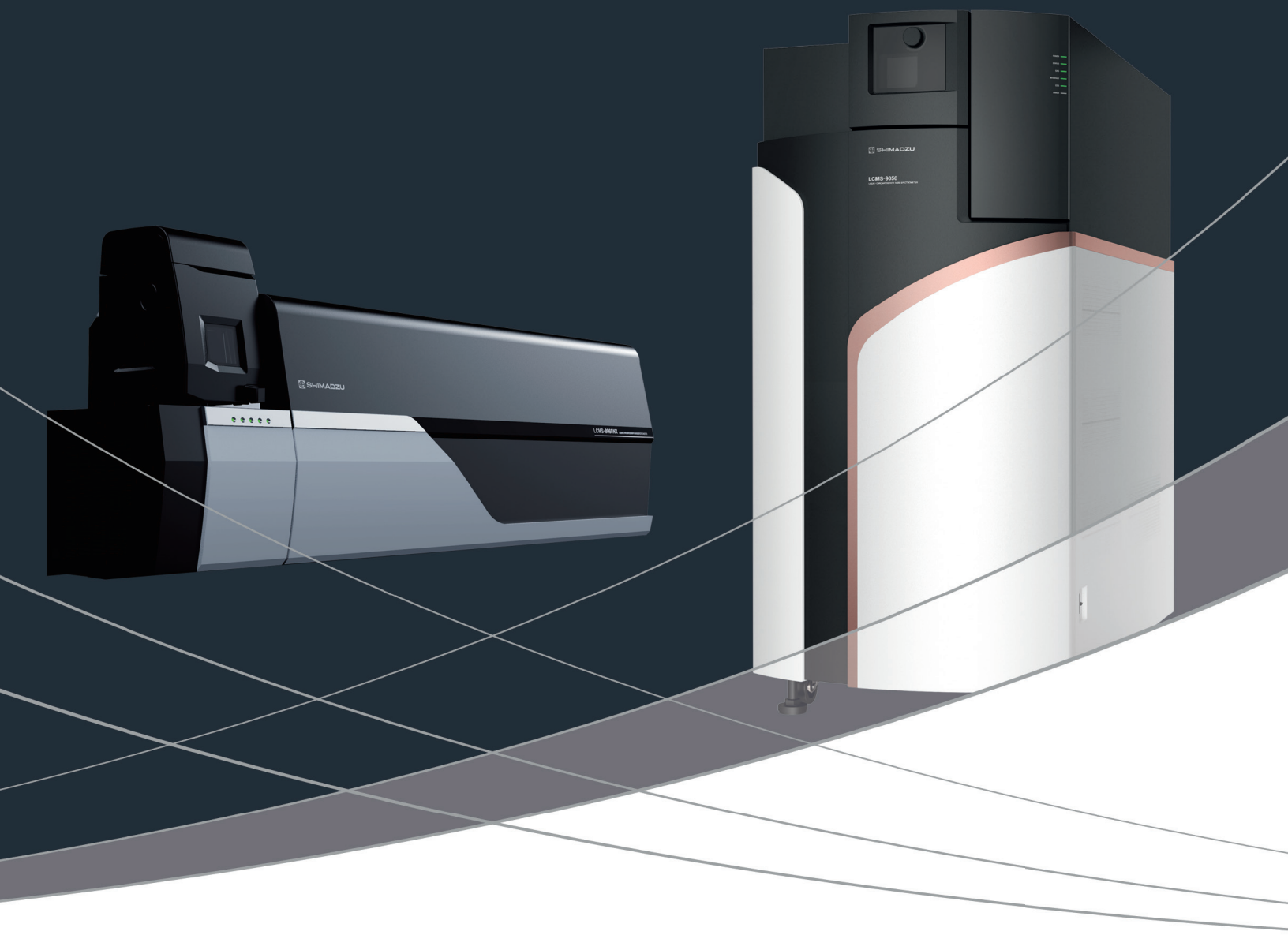

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