PROGRAM AND ABSTRACTS

Hey y'all!

When we began planning the 2021 Annual Meeting of the Society of Forensic Toxicologists, the future was uncertain. SOFTember was a smashing success (thanks in no small part to the efforts of Beth Olson and CC Watson!), but it felt different meeting virtually. We missed the interactions with our toxicology family and remained hopeful that we'd be able to host everyone in person in 2021. It's been a rollercoaster of a year, but the time is here and we are so incredibly excited to welcome you to Nashville!

Our planning committee has put together a great slate of scientific content. Workshops will take place Monday and Tuesday and include topics such as NPS screening, alternative matrices, alcohol calculations, and drug-drug interactions. The scientific program begins on Wednesday morning with our plenary speaker, Dr. Matthew Johnson of the Johns Hopkins Center for Psychedelic & Consciousness Research discussing a hot topic—psychedelics in clinical care. Directly following the plenary, President Amy Miles and Past Presidents Suman Rana and Bruce Goldberger will honor SOFT's Past Presidents with a medallion ceremony. The scientific program will continue through Friday morning.

Are you as excited to catch up with your colleagues as we are? There are a number of opportunities to socialize throughout the week, including Sunday's Young Forensic Toxicologists Symposium, Monday's Thermo Fisher Bluegrass Reception, Tuesday's Welcome Reception and MilliporeSigma Nite Owl Reception, and Wednesday's off-site event at Pinewood Social. Pinewood Social is an indoor/outdoor social club serving craft cocktails and food in a relaxed atmosphere in downtown Nashville. Thursday night's Rhinestone Toxicologists Banquet will be a highlight as we celebrate President Amy Miles and dance to the sounds of the 10-piece Downtown Band.

It's difficult to plan a meeting in a "normal" year, but it's even more challenging when the world changes seemingly overnight. We'd like to acknowledge the hosts of the 2020 Annual Meeting, Denice Teem and Dani Mata, for allowing us to sit in on their planning calls and for modeling fortitude and grace as the word COVID entered our lexicon and their meeting morphed into SOFTember. We'd also like to give a round of applause to our planning committee. In addition to being productive and creative, you've all been very patient and flexible, adapting to changes in the meeting on short notice. We could not do this without you. The SOFT Office, Beth and CC, are truly invaluable—without them, we would be buried in hotel logistics, floor plans, schedules, and even more emails. To the SOFT Board, in particular, President Amy Miles and President-Elect Robert Sears, thank you for your guidance and for helping us ensure that SOFT Nashville has appropriate procedures in place for a safe meeting. Finally, we want to thank you – the members, attendees, presenters, and exhibitors who make this whole thing possible.

While we are excited to meet in person, we need your help to ensure the meeting is as safe as it can be for all attendees. Please do your part by following SOFT's COVID-related guidance. We encourage all attendees to take any additional steps they feel are necessary to safely participate in the meeting. While those who are unable to attend will be missed, we hope you enjoy this opportunity to finally catch up with colleagues and friends in person, learn something new, and visit with exhibitors. Thank you all for helping us make the Nashville meeting a success!

-Jen and Erin

2021 CO-HOST
JEN COLBY, Ph.D., DABCC, FAACC

2021 CO-HOST
ERIN KARSCHNER, Ph.D., F-ABFT

Sunday, September 26, 2021	Time	Place
Registration	10am-5pm	Tennessee Lobby A
NSC-ADID Meeting	10am-1pm	Hermitage C
NLCP Inspector Training	2-6pm	Tennessee B
YFT Symposium	4:30-9pm	Tennessee C

Monday, September 27, 2021	Time	Place
Registration	7am-6pm	Tennessee Lobby A
Continental Breakfast (WS Participants)	7-8:30am	Tennessee Lobby
WS 1: Oral Fluid Testing	8am-12pm	Tennessee B
WS 2: Funding Toxicology	8am-12pm	Tennessee D
Student Enrichment Program	8am-5pm	Hermitage C
ABFT Lab Accreditation Committee	8am-5pm	Magnolia Boardroom B
ABFT Exam Committee	8am-5pm	Magnolia Boardroom A
WS 3: Impact of Cannabinoids	8am-5:30pm	Tennessee C
WS 4: Screening of NPS	8am-5:30pm	Tennessee A
Refreshment Break (WS Participants)	10am-10:30am	Tennessee Lobby
Thermo Fisher Lunch and Learn	12-1:30pm	Ryman Ballroom F
Immunalysis Lunch and Learn	12-1:30pm	Ryman Ballroom AB
Agilent Lunch and Learn	12-1:30pm	Ryman Ballroom DE
Sciex Lunch and Learn	12-1:30pm	Cheekwood ABC
WS 5: Alcohol Calculations	1:30pm-5:30pm	Tennessee B
Refreshment Break (WS Participants)	3:30-4pm	Tennessee Lobby
Designer Drugs Committee	5:30-6:30pm	Belle Meade CD
SOFT-AAFS Drugs & Driving	5:30-6:30pm	Davidson A
Thermo Fisher Bluegrass Reception	7:30-10:30pm	Magnolia Ballroom

Tuesday, September 28, 2021	Time	Place
Continental Breakfast (WS Participants)	7-8:30am	Tennessee Lobby
SOFT Board Meeting	7am-12pm	Magnolia Ballroom
Registration	7am-6pm	Tennessee Lobby A
WS 7: Alternate Matrices	8am-12pm	Tennessee B
WS 8: Poisons	8am-12pm	Tennessee A
WS 9: Drug Interactions	8am-12pm	Tennessee C
ABFT Exam	8am-12pm	Hermitage E
WS 10: Therapeutics	8am-5:30pm	Tennessee D
Refreshment Break (WS Participants)	10-10:30am	Tennessee Lobby
ABFT Laboratory Director Meeting	10-11am	Davidson A
Thermo Fisher Lunch and Learn	12-1:30pm	Ryman Ballroom F
Immunalysis Lunch and Learn	12-1:30pm	Ryman Ballroom AB
Agilent Lunch and Learn	12-1:30pm	Ryman Ballroom DE
Sciex Lunch and Learn	12-1:30pm	Cheekwood ABC
UCT Lunch and Learn	12-1:30pm	Hermitage D
Shimadzu Lunch and Learn	12-1:30pm	Hermitage AB
Randox Lunch and Learn	12-1:30pm	Hermitage C
ConEd Committee Meeting	12:15-1:15pm	Belmont B
ABFT Board Meeting	1-5pm	Magnolia Boardroom
WS 11: Vaping	1:30-5:30pm	Tennessee C
WS 12: Method Validation	1:30-5:30pm	Tennessee B
WS 13: Collaboration	1:30-5:30pm	Tennessee A
Refreshment Break (WS Participants)	3:30-4pm	Tennessee Lobby
Postmortem Committee Meeting	5:30-6:30pm	Belmont B
Welcome Reception	6:30-9:30pm	Ryman Exhibit Hall

Tuesday, September 28, 2021	Time	Place
Elmer Gordon Forum	8:30-10pm	Magnolia Ballroom
MilliporeSigma Nite Owl Reception	10pm-12am	Ryman Ballroom

Wednesday, September 29, 2021	Time	Place
First-Time Attendee Breakfast	7-8am	Tennessee Ballroom D
Continental Breakfast	7-8:30am	Tennessee Lobby
Poster Presentation Prep	7-11am	Ryman Exhibit Hall
Registration	7am-5pm	Tennessee Lobby A
Opening Plenary Speaker	8-9am	Tennessee Ballroom
Presidential Medallion Ceremony	9-9:30am	Tennessee Ballroom
Scientific Session #1	9:30-10am	Tennessee Ballroom
Exhibit Hall	9:30am-4pm	Ryman Exhibit Hall
Refreshment Break	10-10:30am	Ryman Exhibit Hall
Scientific Session #2	10:30am-12pm	Tennessee Ballroom
Lunch with Exhibitors	12-2pm	Ryman Exhibit Hall
Poster Session #1	12-2pm	Ryman Exhibit Hall
SOFT 2022 Planning Committee	12:30-1:30pm	Hermitage C
Scientific Session #3	2-3:30pm	Tennessee Ballroom
Refreshment Break	3:30-4pm	Ryman Exhibit Hall
Scientific Session #4	4-5pm	Tennessee Ballroom
Oral Fluid Committee	5-6pm	Hermitage C
Pinewood Social Off-Site Event	6:30-9:30pm	Pinewood Social

Thursday, September 30, 2021	Time	Place
Karla Moore Fun Run	6:30-8am	Magnolia Portico
Continental Breakfast	7-8:30am	Tennessee Lobby
AAFS Steering Committee	7-9am	Hermitage C
Poster Presentation Prep	7-11am	Ryman Exhibit Hall
Registration	7am-1pm	Tennessee Lobby A
Scientific Session #5	8-9:30am	Tennessee Ballroom
Exhibitor Feedback Meeting	8:30-9:30am	Hermitage D
Refreshment Break	9:30-10am	Ryman Exhibit Hall
Exhibit Hall	9:30am-3:30pm	Ryman Exhibit Hall
Scientific Session #6	10am-12pm	Tennessee Ballroom
Lunch with Exhibitors	12-2pm	Ryman Exhibit Hall
Poster Session #2	12-2pm	Ryman Exhibit Hall
DFC Committee	12:30-1:30pm	Hermitage C
Scientific Session #7	2-3pm	Tennessee Ballroom
Refreshment Break	3-3:30pm	Ryman Exhibit Hall
SOFT Business Meeting	3:30-5pm	Tennessee Ballroom
Happy Hour	6-7pm	Tennessee Lobby
President's Banquet	7-8:30pm	Tennessee Ballroom
Live Band and Dancing	8:30pm-12am	Tennessee Ballroom

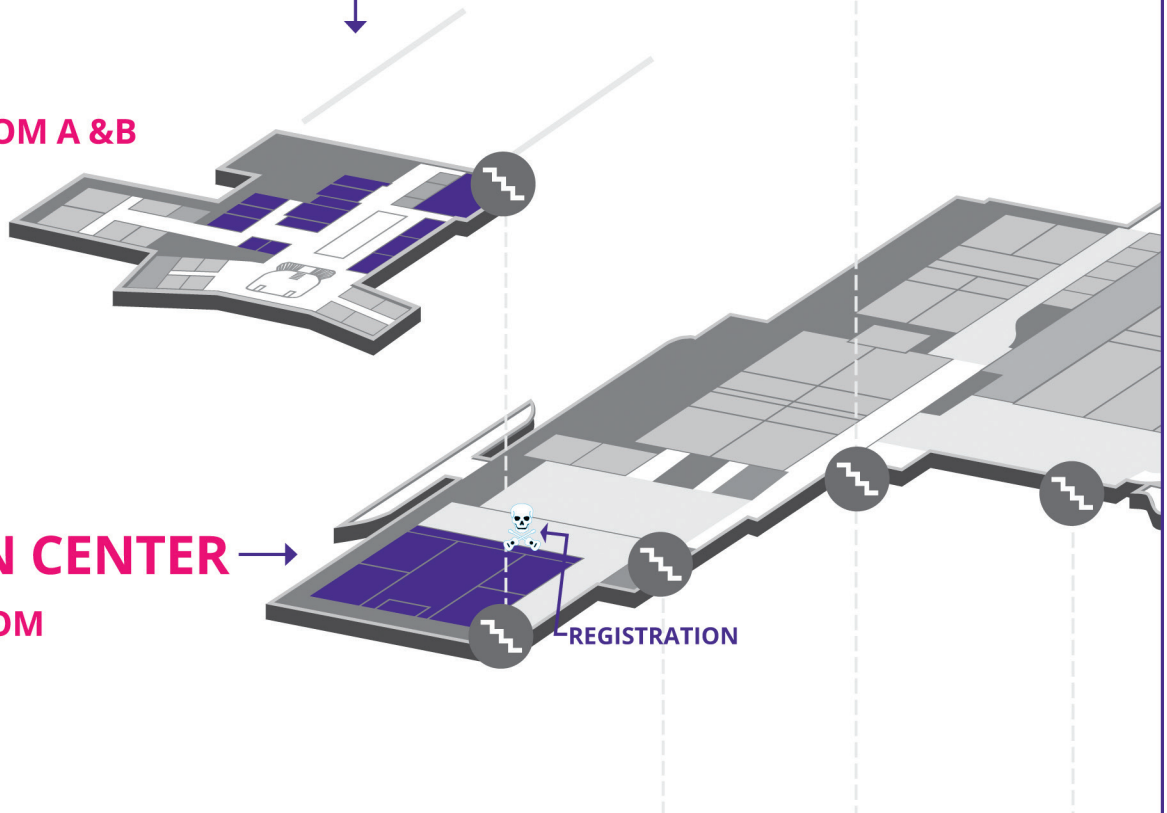| Friday, October 1, 2021	Time	Place
Continental Breakfast	7-8:30am	Tennessee Lobby
Scientific Session #8	8-10am	Tennessee Ballroom
Refreshment Break	10-10:30am	Tennessee Lobby
Scientific Session #9	10:30am-12pm	Tennessee Ballroom
Closing Ceremony	12-12:30pm	Tennessee Ballroom

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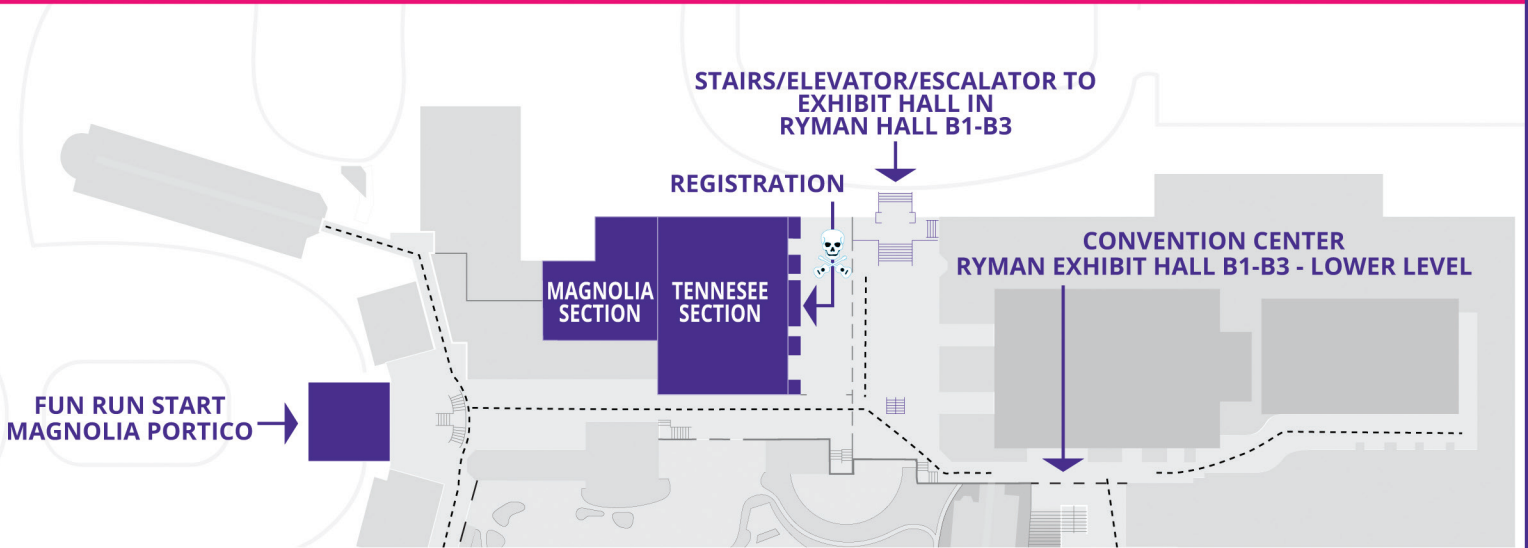
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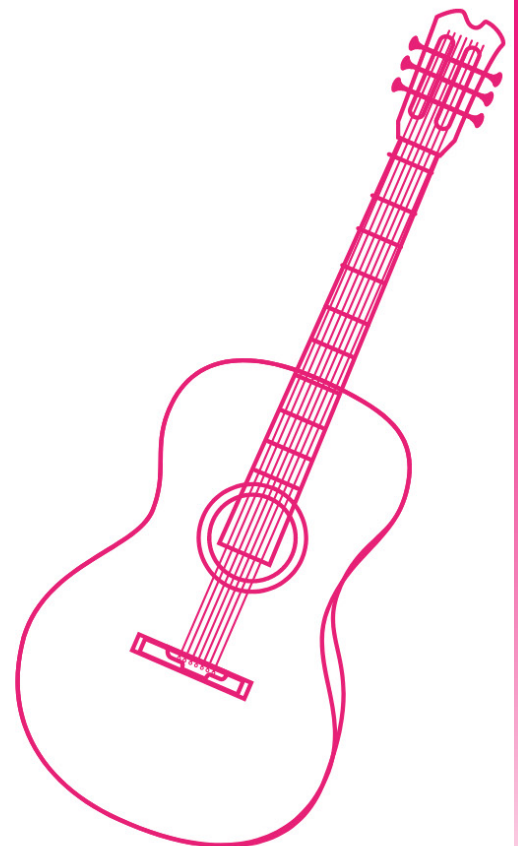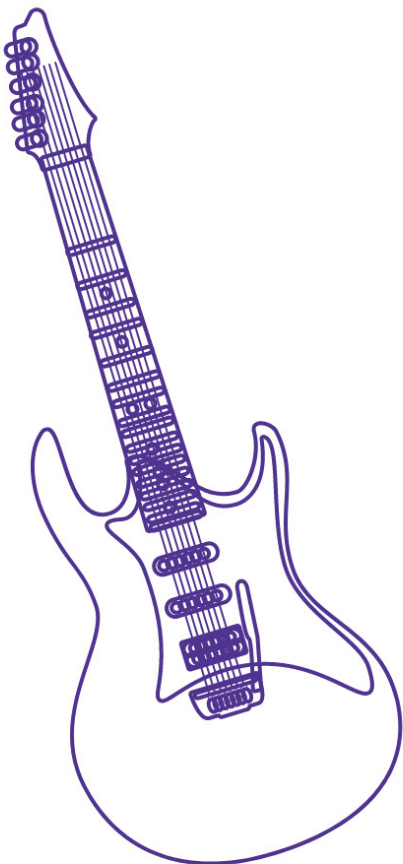**FUN RUN COORDINATORS**
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SOCIETY OF FORENSIC TOXICOLOGISTS
YOUNG FORENSIC TOXICOLOGISTS

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 will be announced at the President's Banquet on Thursday evening.

Symposium and Professional Development Fair (PDF)
Sunday, September 26
4:30-9:00 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, academic and career opportunities including scientific writing. Attendees must be 41 years of age or under to participate in the Symposium.

Agenda

4:30-5:30 pm Professional Development Fair/Social Networking
5:30-5:40 pm YFT Committee Introductions
5:40-6:25 pm Symposium/PDF Speaker: H. Chip Walls - Accessing Journal Articles
6:25-6:35 pm SOFT Board Introductions
6:35-7:15 pm Icebreaker/Professional Mentoring Program Update
7:15-7:30 pm Break
7:30-8:00 pm 2018 Leo Dal Cortivo Award Winner - Sandra C. Bishop-Freeman
              2019 Leo Dal Cortivo Award Winner - Britni Skillman
8:00-9:00 pm Open Forum Discussion

Student Enrichment Program (SEP)
Monday, September 27
8:00 PM – 5:00 PM

The YFT Committee hosts a day-long Student Enrichment Program (SEP) targeting undergraduates and graduate students interested in forensic toxicology. Students will learn about various disciplines within forensic toxicology and what knowledge and skills are necessary for this career path from practicing forensic toxicologists.

Leo Dal Cortivo Award
Thursday, September 31
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 2021 winners will be announced at the President's Banquet on Thursday, September 30.

H. CHIP WALLS

H Chip Walls received his B.S. from the University of Alabama at Birmingham in 1972 during which time he was a research associate in the Department of Chemistry. His professional career has covered more than 45 years including 17 years at the University of Miami Miller School of Medicine, and 21 years with the Alabama Department of Forensic Sciences-Birmingham Division toxicology section and Onondaga County Medical Examiner's Office toxicology laboratories. His experience encompasses post-mortem forensic toxicology, clinical toxicology, probation urine drug testing, drug facilitated crimes and driving under the influence cases. Currently, he is the Chief Wizard at the Forensic Analytical & Clinical Toxicology Laboratory Consulting & Training Specialists in Miami. In addition, he is a technical consultant to the forensic chemistry section of the chemistry department at Florida International University, Miami Police Department Police Academy adjunct instructor and Subject matter expert for police departments in Miami-Dade County serving the Impaired Drivers program.

An active member of several toxicology organizations, he has chaired national committees and has organized numerous workshops on many aspects of the principals and practices of forensic toxicology for annual meetings of various professional organizations. He has been an invited speaker, nationally as well as internationally, on drug detection in pregnancy, the role of toxicology in prosecuting impaired drivers, and information resources in forensic toxicology, marijuana, antidepressants, anti-epileptics, psychotropic medications, narcotics, sedative-hypnotics, Field Impairment Testing and Driving Under the Influence of Intoxicants and alcohol. He has had work published on such topics as cocaine, marijuana, and benzodiazepines and forensic toxicology in peer-reviewed scientific journals or books, and presented at national forensic science meetings.

He has served the Society of Forensic Toxicologist (SOFT) as Past-President (1997), President (1996), Vice-President (1995), Board of Directors (1991-1994), the Executive Board (1995). In addition, he has served on or chaired the following committees: Driving Under the Influence of Drugs (Chair), Meeting Resources (Chair), Joint Committee on Education and Training in Toxicology (JCETT) and Health/Safety. He was SOFT Special Issue Guest Editor of the Journal of Analytical Toxicology (1992). In 2006, he was presented the Ray Abernathy award by the American Academy of Forensic Sciences Toxicology section as "Recognition of an Outstanding Forensic Toxicology Practitioner". In 2009, he was recognized as DRE Ambassador by the Drug Evaluation and Classification (DEC) Program of the International Association of Chiefs of Police. In 2012, he was recognized by Miami-Dade MADD with it highest honor the "Heart of MADD" award for his dedicated service to public safety endeavors; In 2017, he was recognized by the National Highway Safety Administration, one of sixteen in the United States, for his toxicological expertise while serving the public safety community in their efforts remove the impaired driver from our roadways.

He is a member of the executive board of the National Safety Counsel's Committee on Alcohol and other drugs now known as The NSC Alcohol, Drugs and Impairment Division. His eccentricity is widely known to fellow toxicologists concerning his collection of information about the principals and practices in the field of analytical, clinical, and forensic toxicology.

He serves as a laboratory inspector for the National Laboratory Certification Program: Federal Workplace Forensic Urine Drug Testing 1990 to Present (SAMSHA).

YFT FEATURED SPEAKER

DR. KARLA MOORE

Join fellow SOFT attendees on Thursday, September 30, from 6:30-8 am for the 23rd Annual Karla Moore Memorial Tox 'N Purge Fun Run/Walk!

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 exhibitors.

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MATTHEW W. JOHNSON, PH.D.

PLENARY SPEAKER

Dr. Matthew W. Johnson, Ph.D., is Professor of Psychiatry and Behavioral Sciences at Johns Hopkins. He is one of the world's most published scientists on the human effects of psychedelics, and has conducted seminal research in the behavioral economics of drug use, addiction, and risk behavior. Dr. Johnson earned his Ph.D. in experimental psychology at the University of Vermont in 2004.

Working with psychedelics since 2004, Dr. Johnson published psychedelic safety guidelines in 2008, helping to resurrect psychedelic research. As Principle Investigator he developed and published the first research on psychedelic treatment of tobacco addiction in 2014. Dr. Johnson and colleagues published the largest study of psilocybin in treating cancer distress in 2016. His 2018 psilocybin abuse liability review recommended placement in Schedule-IV upon potential medical approval. He is Principle Investigator on funded studies investigating psilocybin in the treatment of opioid dependence and PTSD. Beyond psilocybin, in 2011 Dr. Johnson published the first-ever blinded human research showing psychoactive effects of salvinorin A, the active constituent in Salvia divinorum. He also published in 2017 the first data indicating that MDMA pill testing services may reduce harm, specifically by reducing drug consumption of unknown or undesired adulterants.

Dr. Johnson is recognized for his research in behavioral economics, behavioral pharmacology, and behavior analysis. He has conducted seminal and widely cited research applying behavioral economic principles such as delay discounting and demand analysis to decision making within addiction, drug consumption, and risk behavior. This includes research determining delay discounting to be a fundamental behavioral process underlying addiction across drug classes, using economic demand analysis to determine the roles of nicotine and nonpharmacological factors in the abuse liability tobacco and other nicotine products, and using delay discounting, probability discounting, and demand analysis to understand sexual risk including condom non-use in casual sex situations. He conducted the first research administering cocaine to humans in determining that cocaine increases sexual desire and affects sexual decision making. He has conducted similar research administering methamphetamine and alcohol, examining effects on sexual decision making. He has published studies on drugs across nearly all psychoactive classes, including studies of cocaine, methamphetamine, tobacco/nicotine, alcohol, opioids, cannabis, benzodiazepines, psilocybin, dextromethorphan, salvinorin A, GHB, caffeine, and cathinone analogs compounds (so-called "bath salts").

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Dr. Johnson was 2019 President of the Psychopharmacology and Substance Abuse Division of the American Psychological Association, and is current President of the International Society for Research on Psychedelics, an organization he founded with colleagues. He has received continuous NIH funding as Principal Investigator since 2009. He has reviewed for >75 journals and has served as guest editor on two special issues on psychedelics. Dr. Johnson has reviewed grants for NIH, NSF, the US Military, and multiple governments outside of the US. He is a standing member of the Addictions Risks and Mechansims (ARM) NIH study section. He has provided invited presentations in 13 nations.

Dr. Johnson has been interviewed widely by media about psychedelics and other drugs. These have included interviews by the New York Times, the Washington Post, the Wall Street Journal, the Globe and Mail, the Daily Mail, USA Today, the Chicago Tribune, the San Francisco Chronicle, the Denver Post, the Baltimore Sun, CNN, CBS News, NBC News, the Atlantic, Newsweek, Vanity Fair, Marie Claire, Vogue, Whole Living, the Washingtonian, Scientific American, Nature, Vice, Insider, Inverse, Healthline, and Psychology Today. Dr. Johnson has appeared for interviews on numerous television and radio shows including 60 Minutes, CNN's Wolf Blitzer Situation Room, Fox Business News' Kennedy, the Dr. Oz Show, PBS' Retro Report, Labyrint (television show in the Netherlands), Spectrum News NY1, the BBC World Service, NPR's Morning Edition, NPR's Kojo Nnamdi Show, New Zealand Radio, and Newstalk Radio Ireland. Dr. Johnson's panel discussion with Tim Ferriss at the Milken Institute Global Conference was broadcast on the Tim Ferriss Podcast. Dr. Johnson and his research were featured in an episode of Breakthrough on the National Geographic Channel, produced by Ron Howard, and in Michael Pollan's best-selling book, How to Change Your Mind: What the New Science of Psychedelics Teaches Us About Consciousness, Dying, Addiction, Depression, and Transcendence.

PLATFORM PRESENTATION SCHEDULE
Wednesday, September 29, 2021

Time	Event
8:00-9:00 am	Opening Ceremony
9:00-9:30 am	Presidential Medallion Ceremony

SCIENTIFIC SESSION 1
Tennessee Ballroom
Moderators: Alex Krotulski & Michelle Peace

Time	Session Number	Title	Speaker
9:30-9:45 am	S-001	The SOFT Professional Mentoring Program: To Mentee(or) or Not To Mentee(or)?	Andre Sukta
9:45-10:00 am	S-002	Case Report: Identification of the Hidden Killer 4-fluoro-3-methyl-α-PVP	Jennifer Hobbs
10:00-10:30 am		*Morning Break*	

SCIENTIFIC SESSION 2
Tennessee Ballroom
Moderators: Daniel Baker & Ruth Winecker

Time	Session Number	Title	Speaker
10:30-10:45 am	S-003	What "R-" You Huffing? A Fatal Mixed Refrigerant Exposure	Amber Raines
10:45-11:00 am	S-004	Case Report: A Mysterious Poisoning with Cyclopropylfentanyl: Homicide or Accident? You be the Judge	M. Elizabeth Zaney
11:00-11:15 am	S-005	Assessing Benzodiazepine Involvement in Drug Overdose Deaths Utilizing the State Unintentional Drug Overdose Reporting System, 41 States and the District of Columbia, January 2019-June 2020	Bruce Goldberger
11:15-11:30 am	S-006	Losing Sleep in North Carolina over Pediatric Exogenous Postmortem Melatonin	Sandra C. Bishop-Freeman
11:30-11:45 am	S-007	Analysis of Hydrazoic Acid Using Headspace-Gas Chromatography/Mass Spectrometry and Application to an Unusual Suicide Case	Erin C. Strickland
11:45 am-12 pm	S-008	Quantitation of blood total nitrate and nitrite concentrations in 145 postmortem cases	Ayako Chan-Hosokawa
12:00-2:00 pm		*Lunch*	

SCIENTIFIC SESSION 3
Tennessee Ballroom
Moderators: Wayne Lewallen & Dani Mata

Time	Session Number	Title	Speaker
2:00-2:15 pm	S-009	Highly precise estimates of drug stability in urine using very large data sets	Svante Vikingsson
2:15-2:30 pm	S-010	Comprehensive Drug Screening Using Supported Liquid Extraction (SLE) and Liquid Chromatography Quadrupole Time-of-Flight Mass Spectrometry (LC-QTOF-MS)	Jessica Ayala
2:30-2:45 pm	S-011	A Forward-Thinking Approach to Tackling New Synthetic Opioid "Nitazene" Analogues by Liquid Chromatography Mass Spectrometry	Sara E. Walton

Time	Session Number	Title	Speaker
2:45-3:00 pm	S-012	Analysis of Ethanol Aerosolized in an Electronic Cigarette by Headspace Gas Chromatography-Flame Ionization Detector (GC-FID) and Particle Size Analysis	Erica Sales
3:00-3:15 pm	S-013	Quantification of Fentanyl Analogs in Oral Fluid using LC-QTOF-MS	Kaitlyn Palmquist
3:15-3:30 pm	S-014	The Quantitation of Eutylone in Sexual Assault Cases using LC-MS/MS	Christopher Shanks
3:30-4:00 pm		*Afternoon Break*	

SCIENTIFIC SESSION 4

Tennessee Ballroom

Moderators: Kristin Kahl & Sarah Kerrigan

Time	Session Number	Title	Speaker
4:00-4:15 pm	S-015	Effective and Rapid Workflow for Blood and Urine Testing in Drug Facilitated Crimes Casework	Luke N. Rodda
4:15-4:30 pm	S-016	The Road to a National Standard for the Scope and Sensitivity for Testing Urine in Investigations of Drug-Facilitated Crimes	Madeline A. Montgomery
4:30-4:45 pm	S-017	Synthetic Cannabinoids in Sexual Assault Casework	Jeffrey P. Walterscheid
4:45-5:00 pm	S-018	Toxicological Analysis of Paired Blood and Urine Specimens in Drug Facilitated Crime Cases	Kristin Kahl

Thursday, September 30, 2021

SCIENTIFIC SESSION 5

Tennessee Ballroom

Moderators: Kayla Ellefsen & Suman Rana

Time	Session Number	Title	Speaker
8:00-8:15 am	S-019	Examining the Evidence on Fluorofentanyl – Multidisciplinary Evaluation of this Emerging Drug with a Focus on Forensic Toxicology Investigations	Alex Krotulski
8:15-8:30 am	S-020	Phenethyl-4-ANPP: a marginally active byproduct suggesting a switch in illicit fentanyl synthesis routes	Christophe Stove
8:30-8:45 am	S-021	In vitro functional characterization of a panel of non-fentanyl opioid new psychoactive substances	Marthe Vandeputte
8:45-9:00 am	S-022	NPS Discovery: Evolution of Novel Synthetic Opioids in the United States with a Focus on Metonitazene	Barry K. Logan
9:00-9:15 am	S-023	Emergence of the Novel Opioid, Metonitazene, in Postmortem Toxicology and Detection by LC-QToF-MS and LC-MS/MS	Kevin G. Shanks
9:15-9:30 am	S-024	Case Series Involving Novel Cinnamylpiperazine Synthetic Opioids: 2-Methyl AP-237 and AP-238	Melissa Fogarty
9:30-9:45 am	S-025	The United Nations Office of Drugs and Crime's (UNODC) Early Warning Advisory Toxicology Portal and Expanding Your Laboratory's Scope of Analysis for Novel Psychoactive Substances.	Karen Hart Valencia
9:45-10:00 am	S-026	Designer Benzodiazepines -*In Vitro* Experiments and Toxicological Interpretation of Authentic Forensic Investigations	Carolina Noble

Time		Morning Break
10:00-10:30 am		*Morning Break*

SCIENTIFIC SESSION 6

Tennessee Ballroom

Moderators: Marilyn Huestis & Madeleine Swortwood

Time	Session Number	Title	Speaker
10:30-10:45 am	S-027	Drug Recognition Expert (DRE) Evaluations Involving Synthetic Cannabinoids	Amanda Mohr
10:45-11:00 am	S-028	The Effects of a Global Pandemic on Impaired Driving Trends in Southern Nevada	Timothy Fassette
11:00-11:15 am	S-029	Popular Drug Combinations in DUID Investigations 2018-2020	Jolene J. Bierly
11:15-11:30 am	S-030	Toxic or tolerant? A look at high drug concentrations in six impaired driving cases in Houston	Sara Dempsey
11:30-11:45 am	S-031	Same Night, Different THC DUI	Stephanie Olofson
11:45 am-12 pm	S-032	A Case of Mistaken (Drug) Identity	Erin Spargo
12:00-2:00 pm		*Lunch*	

SCIENTIFIC SESSION 7

Tennessee Ballroom

Moderators: Justin Poklis & Michael Truver

Time	Session Number	Title	Speaker
2:00-2:15 pm	S-033	A Determination of the Aerosolization Efficiency of Drugs of Abuse in a Eutectic Mixture with Nicotine in Electronic Cigarettes	Laerissa Reveil
2:15-2:30 pm	S-034	Impact of Vaping Ethanol on the Roadside Field Sobriety Test in a Clinical Setting	Alaina Holt
2:30-2:45 pm	S-035	Mitragynine: A Postmortem Case Study Perspective (2018-Present)	William Schroeder
2:45-3:00 pm	S-036	Analysis of Umbilical Cord Tissue as an Indicator of In Utero Exposure to Toxic Adulterating Substances	Kari M. Midthun
3:00-3:30 pm		*Afternoon Break*	

Friday, October 1, 2021

SCIENTIFIC SESSION 8

Tennessee Ballroom

Moderators: Marta Concheiro & Tim Rohrig

Time	Session Number	Title	Speaker
8:00-8:15 am	S-037	Postmortem investigations in a death involving anabolic steroid: hair is the key specimen	Pascal Kintz
8:15-8:30 am	S-038	Postmortem Oral Fluid Drug Testing in Alabama	Curt E. Harper
8:30-8:45 am	S-039	Identification and quantification of ketamine derivatives 2-fluorodeschloroketamine, deschloro-N-ethyl-ketamine, deschloroketamine and ephenidine, along with clonazolam, etizolam and other NPS in seized materials and hair: A French case-study	Amine Larabi

Time	Session Number	Title	Speaker
8:45-9:00 am	S-040	Placenta as an alternative matrix to detect cannabis exposure during pregnancy	Marta Concheiro
9:00-9:15 am	S-041	Interlaboratory Comparison of Blinded Hair Testing Reference Samples	Ruth Winecker
9:15-9:30 am	S-042	Evaluation of Extraction Parameters for the Analysis of Three Authentic Hair Reference Materials (HRM) Using Statistical Design of Experiments (DoE)	Brianna Spear
9:30-9:45 am	S-043	Effects of Fentanyl on Blow Fly Development and Quantitation of Fentanyl from Insect Tissue via QuEChERS Extraction and LC-MS/MS Analysis	Joseph Cox
9:45-10:00 am	S-044	Determination of Cannabinoids in Meconium	Carrol Nanco
10:00-10:30 am		*Morning Break*	

SCIENTIFIC SESSION 9
Tennessee Ballroom
Moderators: Jen Colby & Chip Walls

Time	Session Number	Title	Speaker
10:30-10:45 am	S-045	LC/QTOF-MS Analysis of Blood and Oral Fluid Submissions in DUID Cases	Robert M. Lockwood
10:45-11:00 am	S-046	Tianeptine: Antidepressant or Novel Psychoactive Substance (NPS)?	Daniel S. Isenschmid
11:00-11:15 am	S-047	Pharmacokinetic Profile of Δ9-Tetrahydrocannabinol (THC), Cannabidiol (CBD), and Metabolites in Whole Blood Following Vaporization and Oral Ingestion of CBD Products.	Ruth Winecker
11:15-11:30 am	S-048	Xylazine Prevalence in a Chronic Pain and Behavioral Health Population	David Schwope
11:30-11:45 am	S-049	Urinary pharmacokinetics of immediate and controlled release oxycodone and its phase I and II metabolites using LC-MS/MS	Michael T. Truver
11:45 am-12 pm	S-050	A Case Series Of N-ethylhexedrone Intoxications	Bertrand Brunet

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POSTER PRESENTATION SCHEDULE

Wednesday, September 29, 2021

Poster Presentation Prep, 7-11 am

POSTER SESSION #1, 12-2 pm

Ryman Exhibit Hall

Moderators: Regina Aksanov, Theresa Hippolyte, Tonya Mitchell, Carolina Noble

Poster Number	Title	Speaker
P-001	SAMHSA Drug Panel Screening in Oral fluid: Development of screening method at 8 seconds per sample using LDTD-MS/MS	Serge Auger
P-002	High-Throughput Screening Method in Hair Sample: Development of a Screening Method at 8 seconds per Sample using LDTD-MS/MS	Serge Auger
P-003	Detection of Δ9-Tetrahydrocannabinol and Metabolites in the Meibomian Lipids of Tear Samples Through LC-MS/MS	Allen J. Mello Jr.
P-004	Semi-quantitative multiplex screening of multiple drugs from a single urine sample in less than 30 minutes on the biochip analyser Evidence MultiSTAT	P. Greiss
P-005	Method Validation for the Identification of Gamma-Hydroxybutyrate (GHB) in Blood, Urine and Vitreous by GC-MS/MS	Tanuja Sathiraj
P-006	Comparison of *In Vitro* Systems for the Generation of Drug Metabolites in Forensic Toxicology	Ludmyla S. Tavares
P-007	The rise and fall of isotonitazene and brorphine: two recent stars in the new synthetic opioid firmament	Christophe Stove
P-008	Synthesis, chemical characterization, and μ-opioid receptor activity assessment of the emerging group of 'nitazene' 2-benzylbenzimidazole synthetic opioids	Marthe Vandeputte
P-009	Method Development for Analysis of Cannabis Exposure and Oxidative Stress Biomarkers in Exhaled Breath Condensate and Oral Fluid	Meena Swaminathan
P-010	An Evaluation of Weak Anion Exchange Solid Phase Extraction Cartridges for the Quantitation of PFAS Compounds in Human Biological Matrices	Reshma Gheevarghese
P-011	Considerations in Establishing ISTD Acceptance Criteria for Volatile Quantitation Using Headspace Gas Chromatography/Flame Ionization Detection	Lisa Branch
P-012	Fast Screening Method for Drug Facilitated Sexual Assault Drugs by Direct Mass Spectrometric Analysis of Dried Urine Spots	Marta Concheiro
P-013	Fast Analysis of 28 Benzodiazepines and Metabolites in Hydrolyzed and Non-Hydrolyzed Urine by LC-MSMS	Marta Concheiro
P-014	Untargeted Poisonings of Southern African Wildlife	Judith Rodriguez Salas
P-015	Survey of US Residents and Their Usage of Electronic Cigarettes with Drugs Other Than Nicotine	Alaina Holt
P-016	Ethanol stability from five years of a blind quality control program in blood alcohol analysis	Erika Phung
P-017	Long- and short-term stability of methylphenidate and its metabolites in blood	Christina Smith
P-018	Effective Separation of Cannabinoid Isomers in Blood	Megan Farley
P-019	Overcoming Heterogeneity in Urine Specimens: Avoiding False Negatives caused by Endogenous Inhibitors	John J. Tomashek
P-020	Cutting Down on Confirms with Adjunct Urine Drug Screening using Rapid-Fire-Mass Spectrometry	Larissa Karas

P-021	Enzymatic hydrolysis of >10000 ng/mL of codeine, morphine, ethylmorphine and oxymorphone glucuronides in urine at RT for LC-MS-analysis	Joakim Oxelbark
P-022	Evaluating the Efficacy of Three Beta-Glucuronidase Enzymes for the Detection of Opioids for Forensic Toxicology Urine Testing in Drug Facilitated Crime Investigations	Traci Reese
P-023	Pivot Chart Primer: Experiencing the Joy of Excel's Hidden Gem to Critically Evaluate Forensic Toxicology Productivity	Rebekah Boswell
P-024	Enrichment of Hemoglobin Covalently Adducted at β^{93}Cys by Reactive Xenobiotics as Potential Biomarkers of Drug Exposure	William J. Morrison IV
P-025	Validation of an automated sample preparation technique for the comprehensive screening of biological matrices using liquid chromatography time-of-flight mass spectrometry	Rebecca Wagner
P-026	Assessment of oxycodone and metabolite concentrations in urine and oral fluid in patients in prescription drug monitoring programs	Lixia Chen
P-027	Development and Validation of a GC-FID Method for 20 Different Acidic and Neutral Cannabinoids	Waseem Gul
P-028	High-Capacity Screening for Compounds of Forensic Toxicological Interest Using a Standardized LC-MS/MS System and Method	Kristine Van Natta
P-029	Method Validation for the Simultaneous Quantification of 17 Commonly Encountered Central Nervous System Depressants (CNSD) in Whole Blood by LC-MS/MS	Robert Almeida
P-030	Liquid Chromatography Tandem Mass Spectrometry for the Identification and Quantitation of Kratom Alkaloids	Rachel A. Lieberman
P-031	Quantitative Analysis of Illicit Drugs of Abuse in Human Whole Blood by LC-MS/MS	Shahana Wahab Huq
P-032	Implementing Quantitative Drug Screening Using High-Resolution Mass Spectrometry	Crystal Holt
P-033	Multi-panel detection of drugs and drug metabolites in hair samples using a comprehensive extraction method	Kevin He
P-034	Unusual attempted drug-facilitated sexual assault involving MDMA	Anne-Laure Pelissier-Alicot
P-035	Separation of Four tetrahydrocannabinol isomers: Δ6a/10a, Δ8, Δ9, and Δ10 on a Single Quad LCMS	Rachel Lieberman
P-036	Rapid and automated analysis of drugs of abuse in oral fluid using extraction (XTR) tips	William E. Brewer
P-037	Drugs of Abuse in Urine and Plasma Extracted with Microelution SPE Technology and Analyzed via LC-MS/MS	Ritesh Pandya
P-038	Sevoflurane as an Interferent in HS/GC Analysis of Volatiles	Michael Weaver
P-039	Multi-Target Screening and Quantitative Method Validation of 24 Drugs in Synthetic Urine Using Automated Sample Preparation Coupled Directly to LC-MS/MS	Xiaomeng (Kate) Xia
P-040	Evaluation of Sample Preparation Approaches for the Extraction of Amphetamine, Methamphetamine, MDMA and metabolites from Urine prior to GC/MS Analysis	Katie-Jo Teehan
P-041	Modification and Validation of EMIT Oxycodone Urine Screening Kits for Use with Serum	Kimberly Karin
P-042	Efficient Removal of Beta-glucuronidase Enzyme for Quantitative Analysis of Therapeutic Drugs and Drugs of Abuse in Urine by LC-MS/MS	Nicholas Chestara

Thursday, September 30

Poster Presentation Prep, 7-11 am

POSTER SESSION #2, 12-2 pm

Ryman Exhibit Hall

Moderators: April Bramlage, Kacey Cliburn, Marissa Finkelstein, Alexander San Nicolas

Poster Number	Title	Speaker
P-043	Method Validation for the Identification of Fentanyl, Fentanyl Analogs, and Other Synthetic Opioids in Blood, Liver, and Urine by LC-MS/MS	Britni Skillman
P-044	Rapid Screening of 65 Common Drugs and Drug Metabolites in Urine and Blood Using High-Resolution Mass Spectrometry	Casey Burrows
P-045	Intracranial self-stimulation in rats as a model of abuse potential and a predictive tool for threat assessment of emerging drugs	Tyson Baird
P-046	Do you have a parent present? Desalkylflurazepam detection in the absence of a parent compound	Vanessa Meneses
P-047	QuEChERS Extraction and LC-MS/MS Quantitation of Fentanyl Analogs and Metabolites from Liver Tissue to Support Medicolegal Death Investigations	Joseph Cox
P-048	Quantitation of 22 fentanyl analogs by UHPLC-MS/MS	Richard Gibson
P-049	A Validated Qualitative Method for the Identification of Phenibut in Postmortem Blood and Urine by LC/MS/MS	Crystal Arndt
P-050	A Validated Method for the Quantification of Desmosine in Plasma, Urine, and Formalin-Fixed Autopsied Lung Tissue Specimens by LC-MS-MS	Michael Fagiola
P-051	A mathematical model using long-acting injectable medications to estimate hair growth rate when establishing drug exposure history by segmental analysis	Claire Power
P-052	Now You See It, Now You Don't – The Case of Vanishing Drugs in a Urine Drug Screen	Ricky P. Bateh
P-053	The Impact of Changing Synthetic Cannabinoid Drug Trends in a Forensic Laboratory	Lindsay Glicksberg
P-054	Qualitative Analysis of Drug Impregnated Paper Samples from England and Wales Prisons in 2019 and 2020	Asena Avci Akca
P-055	Detection of Mitragynine and 7-Hydroxymitragynine in Keratinized Specimens	Andre Sukta
P-056	Designer Benzodiazepine Positivity and Co-Positivity in Urine Specimens Received from December 2020 to April 2021	Mindy Shelby
P-057	Fentanyl with a dash of Xylazine?	Helen H. Ha
P-058	Advanced search tools for identification of novel fentanyl analogs detected by HRAM Orbitrap mass spectrometry	Stephanie Samra
P-059	Analysis of Suspected Opioid Overdose Samples for Fentanyl and Fentanyl Related Analogues using High Performance Liquid Chromatography Tandem Mass Spectrometer (LC-MS-MS) and High Performance Liquid Chromatography Quadrupole Time of Flight Mass Spectrometer (LC-QTOF-MS)	Unaiza Uzair
P-060	Evaluation of EtG and EtS alcohol markers in urine and oral fluid after hard kombucha consumption	Sun Yi Li
P-061	Profiling Trace Element Contaminants of Toxicological Interest in Commercially Available CBD Tincture Oils	Tom Gluodenis
P-062	Suicide by Tetrahydrozoline in North Carolina	Erin Hensel

P-063	Fatality from 2-Methyl AP-237 (2-methyl buccinazine), a novel synthetic opioid drug gaining recreational popularity	Kimberly L. Samano
P-064	New Psychoactive Substance (NPS) identification by analyses of oral fluid samples collected at electronic music festivals in Brazil	Kelly Cunha
P-065	Cannabidiol in Acidic Foods	Lawrance D. Mullen
P-066	Reaching New Sensitivity Levels for the Detection of Fentanyl Analogs and Highly Potent Novel Synthetic Opioids in Blood	Pierre Negri
P-067	Development of a UPLC-MSMS method for the identification of 52 Fentanyl analogs and 7 isotopically labelled internal standards	Carl E. Wolf
P-068	Rapid Identification and Quantification of Stimulants in Human Urine Using High-Resolution Mass Spectrometry	Alex Wang
P-069	Identification and Quantitation of Fluorofentanyl in Postmortem Blood	Michael T. Truver
P-070	Comparison of Postmortem Toxicology with Seized Drug Evidence from Death Scenes	Samantha E. Herbick
P-071	Fentanyl and Methamphetamine: The New Speedball in Alabama	Mary Ellen Mai
P-072	Buprenorphine co-positivity with designer benzodiazepines in routine healthcare urine samples	Andrew Holt
P-073	The Effect of Designer Benzodiazepines on ELISA Confirmation Rates	Christina Mendralla
P-074	Analysis of Psilocin and Psilocybin, in Urine Using High-throughput LC-MS/MS	Ravali Alagandula
P-075	Analysis of fatalities involving amphetamine in Jazan, Saudi Arabi	Ibraheem M. Attafi
P-076	Demographics and Vitreous Chemistry Results of Hypothermia Deaths Due to 2021 Winter Storm Uri in Harris County, Texas	Andrew S. Greenwood
P-077	Urine drug screening in the era of designer benzodiazepines: results of three immunoassay platforms and comparison to LC-QTOF-MS	Sara Schreiber
P-078	Detection of Eutylone in Chronic Pain and Behavioral Health Populations	Brooke Petrasovits
P-079	A non-fatal intoxication case with MDMA and alcohol consumption	Laurence Labat
P-080	Sensitive Quantitation of Per-and Poly-Fluoroalkyl Substances (PFASs) in Serum	Devin Keller
P-081	Postmortem Toxicology in Kentucky: Looking Back at 2020	Kevin G. Shanks
P-082	The Ever-Changing Scope of Synthetic Cannabinoids in Toxicology Casework II (2016-2020)	Kevin G. Shanks
P-083	Ritalinic Acid in Urine; Effect of Patient Age	Gregory L. McIntyre
P-084	Amphetamine by Age with Prescriptions	Gregory L. McIntyre

S-001: The SOFT Professional Mentoring Program: To Mentee(or) or Not To Mentee(or)?

James M. (Jim) Burris[1], Amanda Cadau[2], Marisol Castaneto[3], Marta Concheiro-Guisan[4], Lindsay Glicksberg[5], Helen H. Ha[6], Erin L. Karschner[7], Beth Olson[8], Michelle R. Peace[9], Kimberly L. Samano[10], Michael L. Smith[11], *Andre Sukta[12], Courtney M. Wardwell[13]

[1]Central Texas Forensic Toxicology, Austin, TX
[2]New York State Police, Albany, NY
[3]DOD Food Analysis and Diagnostic Laboratory, Joint Base San Antonio, Fort Sam Houston, TX
[4]John Jay College, City University of New York, New York, NY
[5]Southwestern Institute of Forensic Sciences, Dallas, TX
[6] Orange County Crime Lab, Santa Ana, CA
[7]Armed Forces Medical Examiner System, Dover AFB, DE
[8]Society of Forensic Toxicologists, Mesa, AZ
[9]Department of Forensic Science, Virginia Commonwealth University, Richmond, VA
[10]Johnson County Medical Examiner's Office, Olathe, KS
[11]Huestis & Smith Toxicology, LLC, Severna Park, MD
[12]United States Drug Testing Laboratories, Inc., Des Plaines, IL
[13]Virginia Department of Forensic Science, Manassas, VA

Background/Introduction: Building a sustainable mentoring program within SOFT will (i) develop and nurture future leaders of the organization and provide a forum for (ii) one-on-one career advice and (iii) mutually beneficial transfer of knowledge to support/advance the organization and forensic toxicology practices. The committee was expanded from six to thirteen members in 2020 through an internal program participant nomination process. To advance the mission of the program, subcommittees were added to oversee the Outgoing Class, Incoming Class, Web-Based Programming, Meeting-Based Programming and Strategic Planning activities. A participant survey was designed to assess previously identified benefits of mentoring, track progress on goals between pairs, establish metrics and target program improvements year over year.

Objective: Present assessment data on self-reported mentor/mentee program outcomes and discuss the evolution of the Professional Mentoring Program's inaugural year.

Methods: Each mentor/mentee pair completed a document to establish mutually agreed upon expectations and identify desired outcomes and measurable goals for the program. Goals were reviewed by the committee and grouped into categories: career advancement, engagement within SOFT, increased dissemination, soft skills and technical development. Subcategories further delineated common goals and/or outputs. In addition to one-on-one mentoring sessions, the 2019-2020 program offered an AAFS breakfast, webinar coaching series, and a recognition ceremony. Feedback on the program's structure and content were collected via participant surveys. The overall program assessment survey consisted of thirty questions and was designed to have the mentoring pairs 1) categorize their contract goals/activities, 2) specify if/how goals had changed, 3) evaluate the professional impact of goals, 4) rate the value(s) of the mentoring relationship, and 5) answer several open-ended questions.

Results: Seventy-four people registered in the inaugural year as either mentor, mentee, or both, resulting in 41 participant pairs. Assessment responses were received from 22 mentors and 26 mentees, or 68% overall participation. For professional activities and goals, both mentees and mentors self-identified significant SOFT engagement, development of leadership and interpersonal skills, and career advancement. Most responses also indicated significant contributions to data, case or research dissemination whereas technical development results were more evenly distributed across the significant/major, moderate and minor options. The committee was interested in understanding if and how mentoring plans changed over the year, especially considering COVID-19. While 29% of the respondents said that their goals were not achieved or postponed, 43% indicated no change in their stated goals. The next set of questions assessed the value of mentoring and indicated significant benefits including expansion of professional network (78%), transfer of knowledge (74%), encouragement (71%), feedback (71%), growth of talent/innovation (57%), and career coaching (53%). Overall, 92% of survey respondents indicated that the program met their needs. Of the 40% that answered they would not participate again, the most common reason was lack of time (27%), followed by continuing the relationship outside the program (17%) or that their goals were met (8%).

Discussion/Conclusion: Data from the Professional Mentoring Program's inaugural year emphasize the value of formal mentorship above and beyond development of professional and technical growth. Excitingly, both survey and anecdotal feedback have captured the strong outcome of emotional support and coaching that is equally beneficial to both mentor and mentee. The data reinforces mentorship is not a one-size fits all approach; each pair and individual derive satisfaction by different facets of the program. Data collection, analysis and interpretation will continue from programming activities to set the foundation to the Professional Mentoring Program. These data points urge agility and innovation to best serve participants and thoughtfully grow individuals in any stage of their career.

PLATFORM

S-002: Case Report: Identification of the Hidden Killer 4-fluoro-3-methyl-α-PVP

Jennifer Hobbs*, Rebecca DeRienz, Daniel Baker, Rachel Barnett, Amber Raines, Morgan Shuttleworth, Brandon O'linn, Maneesha Pandey MD, and Anahi Ortiz MD

Franklin County Coroner, Franklin County Forensic Science Center Columbus, Ohio

Background/Introduction: A 30-year-old non-hispanic white male was found unresponsive at his workplace and admitted to the hospital in cardiac arrest. He was pronounced shortly after arrival. At autopsy the pathologist noted a 176-pound, well-nourished adult, atraumatic male with significant bilateral frothy pulmonary edema (right lung 930g and left lung 1130g), cardiomegaly (430g), dilated ventricles, and slight cerebral edema. Upon completion of the systematic toxicological analysis scope for the Franklin County Coroner's Office Toxicology Laboratory, no known drugs were found. Further review of the GC/MS full scan library summary reports showed an unknown peak in both the blood and urine solid phase extracts. This report noted a possible α-PVP variant. A positional isomer was identified, and a SIM method was validated for the presence of 4-fluoro-3-methyl-α-pyrrolidinovalerophenone (PVP).

Objectives: To share the identification and quantitation of a novel cathinone in the central Ohio region with the medicolegal and toxicological community. In addition, to demonstrate the importance of keeping search libraries current in order to assist in identifying new drug analytes that fall outside the scope of the toxicology laboratory.

Methods: Previously validated full scan screen methods via solid phase extraction and GC/MS analysis, were run on femoral blood and post-mortem urine. Reference material for the positional isomers 4-fluoro-3-methyl-α-PVP and 3-fluoro-4-methyl- α-PVP were first investigated by full scan GC/MS. Isomeric separation was observed and relative retention time comparison confirmed that 4-fluoro-3-methyl-α-PVP was the analyte of interest. A GC/MS SIM method was created on an Agilent 7890B gas chromatograph equipped with a Restek Rxi-17Sil 20m x 0.18mm ID x 0.18um df column. Ions monitored for 4-fluoro-3-methyl-α-PVP were m/z 126, 137, and 109 at a retention time of 6.44 minutes. Quantitation was completed with α-PVP-d8 internal standard using ions m/z 134, 77, and 105. A six-point calibration range of 10-400ng/mL was found to be linear and within ±10% of target concentrations, giving a correlation coefficient (R2) of 0.998. A 10ng/mL limit of quantitation was set with a limit of detection observed at 5ng/mL.

Results: The initial screening tests on femoral blood via 9-panel ELISA, Volatiles by GC/FID, and basic, acidic, neutral blood and urine extracts analyzed by GC/MS, showed no drugs present within the current laboratory scope. Library search capabilities were updated with reference material received and yielded acceptable full scan identification of 4-fluoro-3-methyl-α-PVP. The analyte specific GC/MS SIM method quantitated at 26ng/mL in gray top femoral blood, 30ng/mL in purple top heart blood, and 20ng/mL in red top vitreous humor. The method further identified analyte presence qualitatively in the urine but was not detected in liver.

Conclusion/Discussion: The decedent's cause of death was determined to be due to the use of drugs, namely, Fluoro-methyl-PVP toxicity, and ruled an accident. Investigational interviews corroborated drug use by the deceased with a preference of research chemicals via the internet. No published literature is available currently and to the authors' knowledge this is the first incident of a fatal death solely caused by this substituted cathinone.

PLATFORM

S-003: What "R-" You Huffing? A Fatal Mixed Refrigerant Exposure

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Background/Introduction: Chlorodifluoromethane (R-22), difluoromethane (R-32), and Pentafluoro-ethane (R-125) are common refrigerants used in cooling systems that can also be sought out for abuse through huffing due to the sense of euphoria that is achieved. These compounds are nonflammable, aliphatic halogenated compounds and the act of huffing them can result in the disturbance of cardiac rhythm, dizziness, loss of consciousness, and even death due to the displacement of oxygen in the lungs. In this case study, a 45-year-old man was found deceased with a tube in his mouth that was connected to an unlabeled refrigerant reclamation tank and details of the scene reflect an autoerotic huffing exposure.

Objectives: To increase awareness of the abuse of cooling system refrigerants and share the knowledge that pure calibrant gases are available for reference standard use. Also, to address the challenges that toxicologists face with refrigerant mixtures and to inform the community how these compounds can be identified using common toxicology laboratory equipment. The authors would also like to reiterate the importance of lung sampling and technique used during autopsy for suspected huffing cases.

Methods: During autopsy femoral blood was collected in a gray vacutainer tube, and lung tissue from the apex of the right and left lungs was collected and placed in headspace crimp top vials for toxicological analysis. Calibrant gases for common cooling system refrigerants were obtained from Gasco Affiliates (Oldsmar, FL) and analyzed for retention time and mass-spectral comparisons. N-Propanol was added to the postmortem samples. The samples were run concurrently with the pure calibrant gases and screened via dual column headspace GC/FID with BAC-1 and BAC-2 columns with the following oven parameters: initial temperature 35^oC until 3.75 minutes, ramping 7.5^oC/minute to 80^oC and holding for 0.25 minutes. Due to coelution of the structurally similar R-compounds by GC/FID, qualitative confirmation with mass spectrometry was required. Headspace gas samples were manually injected after a 15 minute, 70^oC incubation, on an Agilent 6890/5973 GC/MS with a BAC-1 column installed and operated isocratically at 35^oC in EI-full scan mode (m/z 30-500, 32.9-500, and alternatively 45-500). The varying scan ranges allowed for coeluting interferences of common ions to be included/excluded. Further results of routine systematic toxicological analysis are tabulated below. Blood samples were reflex extracted for confirmation of sympathomimetic amines with alkaline solid phase extraction and analyzed via GC/MS-SIM with HFBA derivatization to obtain amphetamine quantitation and ethanol was quantitatively reported (LOQ=0.01 g%) with the previously mentioned dual column GC/FID method.

Results:

Sample	Method	R-22	R-32	R-125	Ethanol	Amphetamine
Femoral Blood	GC/FID	Presumptive	Presumptive	Presumptive	0.209 g%	N/A
	GC/MS	Positive	Not Detected	Not Detected	Positive	99 ng/mL
Lung Tissue	GC/FID	Presumptive	Presumptive	Presumptive	Positive	Testing Not Performed
	GC/MS	Positive	Positive	Positive	Positive	Testing Not Performed

Conclusion/Discussion: The decedents cause of death was determined to be mixed refrigerant toxicity by chlorodifluoromethane (R-22), difluoromethane (R-32), and pentafluoroethane (R-125) and was ruled an accident. The identification of refrigerant mixtures can be challenging for toxicologists due to similar retention times and co-elution but can be made possible with the use of readily available instrumentation in the laboratory (ie. GC/MS, benchtop oven, gas-tight/insulin syringes). The sampling of the lung tissue at the apex of the lung proved to be critical in this analysis and allowed for multiple refrigerants to be identified and reported.

PLATFORM

S-004: Case Report: A Mysterious Poisoning with Cyclopropylfentanyl: Homicide or Accident? You be the Judge

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Background/Introduction: This case involves a wealthy, white female of 54 years of age, who, according to her live-in boyfriend, spent an unremarkable day working, had dinner at home consisting of chicken broth, then lay down on the couch as was her customary routine. She was last seen alive by her boyfriend at 11pm sleeping on the couch. She was later found unresponsive on the couch at approximately 1am the following day. He summoned fire rescue who transported her to the emergency room in cardiac arrest. All lifesaving efforts were unsuccessful and the decedent was pronounced at 1:37am. She was a registered organ donor, therefore bones and tissue were harvested prior to a religious style in-situ autopsy the following morning. Blood, bile, ocular fluid and gastric contents were submitted to the Miami-Dade Medical Examiner Toxicology Laboratory (MDME) for toxicology testing. The decedent was then cremated.

Objective: To present all the investigative information and toxicology findings so the audience may make an informed hypothesis as to what may have transpired in this case.

Method: Postmortem specimens (saphenous vein blood, gastric contents and bile) were extracted using mixed-mode solid phase extraction columns. Initial screening was performed using an Agilent gas chromatograph coupled to a mass selective detector (GC-MSD) Forensic Toxicology Analyzer, SP1 7890-0458 with Deconvolution Reporting Software (DRS). Confirmatory analysis was conducted using a Thermo Scientific Dionex Ultimate 3000 RSLC ultra high-performance liquid chromatograph coupled to a Bruker Daltonics AmaZon Speed™ ion trap mass spectrometer (UHPLC-Ion Trap-MS^n) equipped with Toxtyper™ software. Quantitative analysis was run on a Shimadzu Nexera X2 liquid chromatograph coupled to a Shimadzu 8060 mass spectrometer (LC-MS/MS).

Results: Cyclopropyl fentanyl was the predominant peak in both the GC-MS screen and the UHPLC-Ion Trap-MS^n screening of the saphenous vein blood and was later quantified at 919 ng/mL. The blood also contained methoxyacetyl fentanyl (4.9 ng/mL), despropionyl fentanyl, fentanyl (46 ng/mL), acetyl fentanyl (<1 ng/mL), butyryl fentanyl (<1 ng/mL), morphine (<0.01 mg/L), phentermine, 7-aminoclonazpam, meclizine and quinine. Atropine and amiodarone (administered by fire rescue) were also detected in blood. Analysis of the gastric contents revealed cyclopropyl fentanyl (0.163 mg total), methoxyacetyl fentanyl, fentanyl, phentermine, 7-aminoclonazepam, ibuprofen and amiodarone. Bile tested positive for cyclopropyl fentanyl, fentanyl, morphine and phentermine.

Conclusion/Discussion: The Medical Examiner determined the cause of death to be "acute combined drug toxicity (fentanyl, cyclopropyl fentanyl, methoxyacetyl fentanyl)"; however, the manner of death is most intriguing in this case. How did fentanyl and its analogues end up in this successful middle-aged woman? None of her friends or family knew of any drug abuse history. She was trying to lose weight and was seeing a doctor who had prescribed phentermine which her boyfriend stated was causing her some stomach issues. Otherwise, she was a healthy, dynamic individual who was well known in social circles in Miami and owned her own successful company. Shortly after her death, the actions of her boyfriend began to arouse suspicions. He allegedly withdrew $10,000 on the date of her death, then ten days later filed a petition to probate a will which would make him the sole beneficiary and executor of her estate which was worth more than $1 million. Within a month, he had withdrawn close to $600,000, and was later arrested for will fraud. Seventeen months after her death, while out on bail, he was riding his motorcycle in the Everglades when he collided head-on with a semi-truck. His death was ruled suicide. Cases of accidental overdoses with cyclopropyl fentanyl, fentanyl and other fentanyl analogues are very common in Miami, however this is the first case in our department where there is a suspicion of homicide using these drugs of abuse.

Keywords: Cyclopropyl fentanyl, overdose, manner of death

S-005: Assessing Benzodiazepine Involvement in Drug Overdose Deaths Utilizing the State Unintentional Drug Overdose Reporting System, 41 States and the District of Columbia, January 2019-June 2020

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Background/Introduction: Drug overdose deaths in the United States increased by 4.8% from 2018 to 2019 and provisional data show a further increase in 2020. Opioids contributed to nearly three-quarters of all drug overdose deaths in 2019. Benzodiazepines are central nervous system depressants and are commonly prescribed for the treatment of anxiety disorders, insomnia, seizure disorders, and alcohol withdrawal. There is generally low risk of overdose with benzodiazepine use alone; however, the use of benzodiazepines with opioids can greatly increase the risk of overdose. The CDC Guideline for Prescribing Opioids for Chronic Pain has recommended that clinicians avoid prescribing opioids and benzodiazepines concurrently, but prescription benzodiazepines are often diverted and distributed illicitly. Also, newer illicitly manufactured benzodiazepines have become more available in the illicit drug market.

Objectives: To assess the contribution of benzodiazepines – both prescription and illicitly manufactured – to drug overdose deaths in jurisdictions contributing data to the Centers for Disease Control and Prevention's (CDC) State Unintentional Drug Overdose Reporting System (SUDORS).

Methods: SUDORS funds 47 states and the District of Columbia to abstract drug overdose death data from death certificates and medical examiner/coroner reports, including full postmortem toxicology results. Jurisdictions are funded to report data to SUDORS with a lag of 6-11 or 8-13 months after the date of death. Data on overdose deaths that occurred during January 1, 2019 - June 30, 2020, from 42 of the 48 funded jurisdictions with complete data, were analyzed to determine the number and percentage of drug overdose deaths and opioid-involved overdose deaths, in particular, with benzodiazepines detected (benzodiazepine-positive) and contributing to the overdose death (benzodiazepine-involved). Benzodiazepines were further categorized as prescription or illicitly manufactured; toxicology results for some deaths included a generic "benzodiazepine" that couldn't be categorized as prescription or illicitly manufactured.

Results: During January 1, 2019 - June 30, 2020, a total of 65,869 drug overdose deaths occurred in the 42 jurisdictions, and opioids were involved in 52,865 (80.3%) deaths. Among all drug overdose deaths, 15,258 (23.2%) were benzodiazepine-positive, and 9,775 (14.8%) were benzodiazepine-involved. Prescription benzodiazepines were detected in 13,569 (20.6%) of all drug overdose deaths and involved in 8,532 (13.0%). Illicitly manufactured benzodiazepines were detected in 1,079 (1.6%) of drug overdose deaths and involved in 891 (1.4%). Among opioid-involved overdose deaths, 13,470 (25.5%) were benzodiazepine-positive, and 8,862 (16.8%) were benzodiazepine-involved. Prescription benzodiazepines were detected in 12,051 (22.8%) of opioid overdose deaths and involved in 7,807 (14.8%). Illicitly manufactured benzodiazepines were detected in 997 (1.9%) of opioid overdose deaths and involved in 825 (1.6%). The illicitly manufactured benzodiazepines most commonly detected among all drug overdose deaths were etizolam (541 deaths), flualprazolam (505 deaths), and flubromazolam (33 deaths). The illicitly manufactured benzodiazepines most commonly involved among all drug overdose deaths were etizolam (439 deaths), flualprazolam (427 deaths), and flubromazolam (26 deaths).

Conclusion/Discussion: Benzodiazepines were involved in 14.8% of drug overdose deaths during January 1, 2019–June 30, 2020 and were co-involved in 16.8% of opioid-involved overdose deaths. Among benzodiazepine-involved deaths, nearly 1 in 10 involved one or more illicitly manufactured benzodiazepines. Benzodiazepines have a high potential for misuse and, in combination with opioids and other sedatives, can increase the risk of overdose. Enhanced toxicology testing and surveillance activities should inform prevention and response efforts, especially as newly emerging illicitly manufactured benzodiazepines become increasingly available in the illicit drug market. Such efforts could include raising awareness among clinicians and people who use illicitly manufactured benzodiazepines about the risk of these substances, especially in combination with opioids.

PLATFORM

S-006: Losing Sleep in North Carolina over Pediatric Exogenous Postmortem Melatonin

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Background/Introduction: Melatonin is an endogenous neurohormone, which regulates circadian rhythms and natural sleep. It is structurally related to tryptophan and serotonin. As an endogenous substance, the concentration of melatonin in plasma, peaks during periods of darkness at around 0.09 ng/mL in young adults. Dietary melatonin supplements are widely used as an exogenous hormone to help reduce sleep-related disorders including insomnia, anxiety and jet lag. The North Carolina Office of the Chief Medical Examiner has investigated seven unexplained infant deaths where melatonin was found upon toxicological analysis. While both the cause and the manner of all deaths with detectable melatonin were classified as undetermined, the findings may be noteworthy. Melatonin has been generally considered as a safe, natural product for a wide age range and it appears in a surprising number of over-the-counter preparations geared towards young children to facilitate calmness and sleep. Endogenous melatonin production does not begin until several months after birth, so the use of exogenous supplements can be brought into question for infants. Another complication is the lack of regulatory control of these supplements. Literature studies show not only the variability of melatonin content due to production and/or stability, but also the possible presence of serotonin as a by-product.

Objectives: Nine cases where melatonin was considered in infant deaths will be presented. Melatonin was detected in seven of the nine cases, while the remaining two had melatonin disclosed in the case history. These data, along with case studies from literature, will provide toxicologists, additional knowledge and understanding of melatonin in postmortem casework.

Methods: As an endogenous compound, melatonin is not detected in the routine GC-MS/NPD organic base screen performed at the North Carolina OCME. Even when taken exogenously as a natural product, it is rarely observed in adult postmortem casework as literature values for single oral doses of 10-80 mg given to men result in a peak serum melatonin of 4-33 ng/mL. With an alphaprodine internal standard retention time of approximately 8.8 minutes on the GC-MS, melatonin elutes at approximately 12.2 minutes at a retention time similar to codeine under current instrumental conditions. The quantitation of melatonin in blood was performed by a reference laboratory.

Results:

Case Number	Age/Sex	Fever Reported	Melatonin (ng/mL)	Blood Collection Site	Details surrounding Melatonin
1	3-month-old/F	No	1400	peripheral	Mother gave 5 mg Tablet dissolvable Melatonin
2	2-month-old/M	No	460	heart	Mother claims to be breastfeeding and taking Melatonin
3	3-month-old/M	Yes	170	aorta	106 deg fever, no melatonin information provided
4	6 month old	Yes	82	vena cava	Had cough, diarrhea, fussiness past 3 days
5	3-year-old/M	Yes	73	iliac	Flu-like symptoms, ward of state sharing room with others
6	5-month-old/M	No	10	iliac	Caregivers crushing melatonin for child
7	2-year-old/M	No	3	femoral	Given 3x3mg melatonin tablets at night for sleep
8	6-month-old/M	No	None Detected	N/A	Started liquid melatonin recently prior to death
9	20-month-old/F	Yes	None Detected	N/A	Mother thought neighbor gave melatonin to child

Conclusion/Discussion: Melatonin has been considered a safe dietary supplement, yet the lack of regulation during manufacturing, especially for consumption by children, and recognition of exogenous melatonin appearing in postmortem pediatric casework raises questions. Melatonin is not known to be toxic, however its mechanism of action may induce thermoregulatory effects on the human body. Several of the decedents were feverish at or near their time of death. While the cases are currently considered undetermined, the work presented here will discuss possible implications for the significance of melatonin with a potential link to physiological conditions, such as fever, and/or the potential exacerbation of unsafe sleeping positions prior to death.

PLATFORM

S-007: Analysis of Hydrazoic Acid Using Headspace-Gas Chromatography/Mass Spectrometry and Application to an Unusual Suicide Case

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Background/Introduction: Sodium azide is an inorganic salt that can be used as an explosive or a biological preservative. While most incidental exposures are non-lethal, since 1966 there have been approximately 20 reported suicides and 13 other deaths related to sodium azide. Symptoms of azide toxicity can include vomiting, nausea, loss of consciousness, hypotension, and metabolic acidosis. The mechanism of action is not well understood, but displays similarities to cyanide and inhibits many enzymatic pathways, such as cytochrome oxidase. Azide can be converted in vivo to nitric oxide and hydrazoic acid (HA), which are believed to cause hypotension and metabolic acidosis, respectively. Sodium azide is not a common toxicological target. Previously reported methods include high performance liquid chromatography with diode array detection, capillary electrophoresis, and gas chromatography (GC) with nitrogen phosphorous detection. Presented here is a suicide in which azide was qualitatively identified using a simple headspace GC/mass spectrometry method (HS-GC/MS). A 31-year-old male with a history of depression was found dead in his bedroom next to a glass measuring cup and spoon with an unknown residue, and a plastic container labeled as sodium azide with a residue inside. An autopsy was performed approximately 38 hours after the decedent was last known to be alive, and various biological specimens were collected for toxicological analysis.

Objectives: Present a HS-GC/MS method for the detection of HA in biological specimens and application to a case study.

Methods: Postmortem specimens were analyzed at Harris County Institute of Forensic Sciences for volatiles, vitreous chemistries, and screened for drugs using a 10-panel ELISA screen and GC/MS. A blood sample was sent to NMS Labs for cyanide and methemoglobin analysis, while a separate blood sample and stomach contents (SC) were sent to the FBI Laboratory for azide analysis. Azide qualitative analysis was validated per ASB Standard 036 using a HS-GC/MS, with a DB-624 column. The HS-GC/MS used a temperature program and selected ion monitoring with electron impact ionization. The limit of detection was 2 µg/mL. SC were homogenized with deionized water prior to analysis. Samples, 0.5 mL blood or 1 g homogenate, were prepared in a 10-mL headspace vial by adding 50 µL of internal standard (0.1% 1-butanol) and capped. A plastic syringe was used to add 0.5 mL of 5 N sulfuric acid to the vial and then vortexed before equilibrating for 30 minutes at room temperature to convert the azide to HA prior to HS-GC/MS analysis.

Results: In-house testing was unremarkable with normal vitreous chemistries and no positive drug results. NMS Labs detected 0.40 µg/mL cyanide and 26% methemoglobin saturation. HA was identified in SC by the FBI Laboratory, but not in the blood. The presence of HA is indicative of azide exposure. Additionally, the FBI Laboratory identified sodium azide in the glass measuring cup and the plastic bottle. The case was classified as a suicide due to sodium azide toxicity.

Conclusion/Discussion: The absence of HA in blood is consistent with other reports. This could be related to the extended length of time between sample collection and analysis as it is possible for azide to oxidize after collection to nitric oxide via oxyhemoglobin. The identification of HA in the SC was expected, as previously reported cases had azide detected in gastric contents with or without positive results in blood. This is the first report of HA identification using HS-GC/MS without derivatization. The sample preparation is simple and allows for the analysis of various biological specimens. While the method is qualitative, quantitation is not necessary, as identification in postmortem samples can be sufficiently informative when combined with other case circumstances.

PLATFORM

S-008: Quantitation of blood total nitrate and nitrite concentrations in 145 postmortem cases

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Background/Introduction: Sodium nitrate and sodium nitrite are inorganic compounds found endogenously, in water and some foods. They are also commonly used as preservatives and fertilizers. Although not harmful at low concentrations, death can occur at higher nitrate/nitrite concentrations from acute methemoglobinemia. Postmortem percent MetHb may rapidly change following sample collection, during storage, and due to decomposition and putrefaction, rendering postmortem interpretation complex. With the rise in suicide by sodium nitrate/nitrite ingestion due to easily accessible web-based instructions and purchase of materials, toxicological analysis for nitrates/nitrites has proved to be a preferrable alternative to testing for methemoglobin as a biomarker for sodium nitrates/nitrites exposure.

Objectives: To provide a toxicological method in suspected suicidal ingestion of sodium nitrate/nitrite, the quantitation of total nitrate/nitrite in postmortem bloods was developed and performed using Spectrophotometry.

Methods: An assay to measure total nitrate/nitrite concentration in the sample was developed using a nitrate/nitrite Colorimetric Assay Kit. Whole bloods were first ultrafiltered using pre-rinsed 10 kDa molecular weight cut-off filters in a centrifuge (14,000 x g) for 30 minutes at 4°C. The diluted filtrate (80 μL) was incubated along with enzyme cofactor (10 μL) and nitrate reductase (10 μL) at room temperature for three hours. This step converts nitrate to nitrite, followed by a formation of a purple azo chromophore. Absorbance of the azo compound was measured using a 540 nm wavelength. Validation experiments were carried out to assess linearity, precision, and accuracy. A linear regression fit was used for the standard calibration curve with a correlation coefficient of $R^2 > 0.90$. The acceptance criteria range for the coefficient of variation (CV) was <20% from the target. The concentration of total nitrate/nitrite in blood was determined by extrapolation of the measured absorbance. The total nitrate/nitrite concentrations, percent MetHB, and concomitant findings within 145 postmortem cases reported by NMS Labs from November 2019 to March 2021 were also reviewed.

Results: A baseline concentration for total nitrate/nitrite was calculated to be 34 μM from postmortem whole blood samples of five known unexposed individuals. In total, 156 cases were examined for total nitrate/nitrite concentrations and 145 cases were confirmed positive. Of those, concentrations greater than 1,000 μM were reported in 90% (n=136). The mean and median total nitrate/nitrite blood concentrations in 136 cases (>100 μM) were 6,300 ±3,300 μM and 6,000 μM, respectively (range: 110-20,000 μM). When gender and age were provided, 69% were male. The mean and median ages were 30 and 26 years old, respectively (range: 13-80). When comparing total nitrate/nitrite concentrations and percent MetHb, there was a positive correlation in 47 cases. Of 105 cases in which other drug screen panel was performed, total nitrate/nitrite was the only finding in 28 cases (27%). 20 additional cases were accompanied by one additional finding. Anti-depressants were the most prevalent other findings, followed by metoclopramide and cannabinoids. Of note, qualitative determination of metoclopramide was made in 24 cases (28%; n=85). Metoclopramide, a prescription medication indicated to prevent and treat nausea, emesis, and heartburn due to gastroparesis, is specifically listed on the suicide forums and included in a suicide kit.

Conclusion/Discussion: The number of suicides by nitrate/nitrite ingestion has been on the rise. The routine toxicological analysis typically reveals no other findings except for a significantly elevated concentration of nitrate/nitrite (>1000 **μ**M). If reported, other findings were prescription medications within the therapeutic concentrations. This study also demonstrated that total nitrate/nitrite concentration cannot be estimated based on the percent MetHb in postmortem blood specimen. A direct measurement of total nitrate/nitrite concentration is preferable to obtain analytical verification when scene investigation and/or autopsy examination suspect an involvement of sodium nitrate/nitrite.

S-009: Highly precise estimates of drug stability in urine using very large data sets.

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Background/Introduction: Studies conducted to investigate the stability of drugs are complicated by the fact that significant losses due to instability, frequently are similar to, or sometimes even smaller than, the uncertainty of the method used. One way to compensate for this and other sources of variability is to repeat the study many times, but such designs are costly and might be beyond the capabilities of individual laboratories.

Objectives: The objective of this study was to use the large data sets accumulated during production of reference materials as well as from the National Laboratory Certification Program (NLCP) performance testing (PT) to determine the stability of drugs of abuse in urine.

Methods: Analytes spiked into human (n=455) or synthetic (n=384) urine without preservatives (pH 6-7) were tested when initially prepared (n=9) and after five days of storage (2-8 °C, n=1). The result after storage was compared to the initial mean as a measure of stability. In the NLCP PT program, some samples are sent to laboratories more than once, in different PT sets. The difference in mean from more than 22 laboratories before and after six months of frozen storage (approx. -20 °C) was used as a measure of stability. All testing was conducted by commercial laboratories accredited by ISO 17025, certified by the Department of Health and Human Services (HHS), or demonstrating similar levels of quality. In total, around 14,000 measurements were conducted. Data points were removed if they were more than 1.5 inter-quartile ranges (IQR) below Q1 or above Q3. The mean and standard error of the mean (SEM) were calculated using remaining data points. Instability was determined as a significant (2xSEM) loss compared to the initial concentration.

Results: The results are presented in the table below.

Analyte	5-day stability (2-8 °C)		6-month stability (frozen)	
	n	mean (±2xSEM)	n	mean (±2xSEM)
Analytes included in NLCP performance testing				
6-acetylmorphine	32	101.1% (98.9-103.2)	16	98.5% (97.6-99.5)
Amphetamine	44	98.2% (97.3-99.0)	12	99.8% (99.0-100.6)
Benzoylecgonine	47	100.1% (99.2-101.1)	15	98.7% (98.2-99.2)
Codeine	49	99.7% (97.7-101.7)	11	100.7% (100.0-101.4)
Hydrocodone	58	101.4% (99.8-103.0)	10	99.7% (98.3-101.0)
Hydromorphone	59	99.0% (97.4-100.7)	3	98.6% (97.6-99.6)
Methamphetamine	32	99.5% (98.6-100.5)	13	99.2 (97.6-100.8)
MDA	48	99.1% (97.4-100.8)	10	99.1% (98.2-99.9)
MDMA	47	99.9% (98.6-101.2)	11	99.7% (99.0-100.4)
Morphine	57	99.4% (97.2-101.6)	12	99.4% (98.2-100.7)
Oxycodone	54	101.0% (99.7-102.4)	7	100.3% (100.0-100.7)
Oxymorphone	64	101.9% (100.6-103.2)	3	99.2% (98.1-100.2)
Phencyclidine (PCP)	48	100.1% (98.2-101.9)	7	99.3% (97.6-101.0)
Δ9-THC-COOH	35	98.6% (96.7-100.4)	11	100.4% (99.3-101.5)

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Other common analytes			
Buprenorphine	9	95.4% (90.0-100.9)	
Norbuprenorphine	6	95.1% (88.8-101.4)	
Methadone	8	100.1% (95.5-104.6)	
EDDP	7	97.2% (91.3-103.1)	
Fentanyl	7	105.7% (97.3-114.1)	
Norfentanyl	4	99.6% (90.6-108.6)	
Tramadol	5	99.1% (95.8-102.4)	Not tested
Oxazepam	10	97.5% (94.3-100.8)	
7-aminoclonazepam	7	88.6% (78.1-99.2)	
Nordiazepam	5	96.2% (93.6-98.8)	
Alprazolam	6	101.3% (99.2-103.4)	
Butalbital	6	99.6% (97.5-101.7)	
EtS	12	92.6% (88.5-97.7)	
EtG	9	96.2% (91.0-101.4)	

Conclusion/Discussion: Refrigerated storage at 2-8 °C for five days represents common conditions during processing of urine specimens in forensic laboratories, and it was shown that all drugs were stable during this time. No losses >20% were observed, but the loss of 11.4% seen for 7-aminoclonazepam might be relevant in some laboratories even though it could be acceptable in an ISO17025-accredited laboratory. Only minor losses (<8%) were observed for other drugs. Similarly, six months of frozen storage is relevant considering reanalysis of specimens. Again, only minor losses (<2%) were observed. However, the pH of the samples was 6-7, and some investigated drugs might not be stable under more extreme conditions. In summary, this study illustrates that large data sets can be used to produce highly precise estimates (1-2%) of drug stability during storage.

PLATFORM

S-010: Comprehensive Drug Screening Using Supported Liquid Extraction (SLE) and Liquid Chromatography Quadrupole Time-of-Flight Mass Spectrometry (LC-QTOF-MS)

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Introduction: Immunoassay (IA) has enjoyed widespread use for forensic toxicology screening over many decades. Although it offers sufficient sensitivity, it lacks specificity and presents technical challenges associated with the identification of a growing number of new psychoactive substances. Mass spectrometry (MS)-based techniques can be used to simultaneously identify a larger number of drugs with multiple data acquisition modes and retrospective data analysis. Draft guidelines for the scope and sensitivity of screening and confirmatory testing for drug impaired driving, drug facilitated crimes, and medicolegal death investigations have been published. Blood is the most frequently encountered biological fluid in forensic toxicology. However, the extraction of multiple drug classes using a single protocol can be challenging. As such, many MS-based screening methods do not include cannabinoids, and laboratories still rely on IA based detection. In this report, SLE was used to simultaneously isolate >200 drugs, including 11-nor-9-carboxytetrahydrocannabinol (THCA) from whole blood at the recommended cutoff concentrations for drug impaired driving.

Objectives: Develop and validate a LC-QTOF-MS screening method that meets or exceeds the requirements for drug impaired driving investigations.

Methods: An Agilent 6530 LC-QTOF-MS was used to identify >200 drugs including amphetamines, analgesics, anticonvulsants, antidepressants, antihistamines, antihypertensives, barbiturates, benzodiazepines, cannabinoids, cathinones, dissociatives, fentalogs, hallucinogens, hypnotics/sedatives, muscle relaxants, opioids, and stimulants. Separation was achieved using an Agilent Poroshell 120 EC-C18 (2.1x100mm, 2.7 µm) and gradient elution (mobile phase A: 5mM ammonium formate/0.01% formic acid in water; mobile phase B: 0.01% formic acid in acetonitrile). Tier I drugs included the most commonly encountered substances at the recommended cutoffs in blood. Tier II, included regionally or geographically specific substances, and supplemental compounds "Tier III" were included at forensically relevant concentrations. Drugs of interest in medicolegal death investigations were also included (e.g. salicylate, acetaminophen). Blood (0.6 mL) was diluted with 0.1M acetic acid (0.3 mL) and centrifuged prior to SLE (Biotage SLE+). After five minutes, drugs were eluted with two fractions of 3 mL n-hexane/ethyl acetate/isopropanol (70:23:7, v/v). Following the addition of acidic methanol, the eluent was evaporated to dryness under nitrogen and reconstituted in 20 µL of mobile phase A:B (60:40). Each extract was analyzed using positive and negative electrospray ionization (ESI) using All Ions acquisition.

Results/Discussion: Limits of detection (LOD) for Tier I drugs ranged from 0.5 to 50 ng/mL. All compounds (including challenging analytes, e.g. THCA) were detected at or below the recommended cutoffs. All analytes with the exception of THCA, barbiturates, topiramate, phenytoin, acetaminophen, non-steroidal anti-inflammatory drugs, lamotrigine, valproic acid, and gamma-hydroxybutyric acid were acquired using positive ESI. Matrix effects and detectability for all analytes at the cutoffs were established using ten independently sourced whole blood samples. Ion suppression and enhancement were observed. Retention time, mass accuracy, and coelution scores were used for identification. Extraction efficiencies were highly compound dependent, and the SLE protocol was selectively optimized to increase THCA recovery at the expense of other compounds more readily detected. The lowest extraction efficiencies using the SLE approach were observed for zwitterions (e.g. benzoylecgonine). Carryover, processed sample stability, and interferences were also evaluated, and a total of ten isotopically labelled internal standards were utilized.

Conclusion: Following selective optimization of the SLE protocol, All Ions acquisition using positive and negative ESI was used to identify >200 drugs at forensically relevant concentrations in whole blood. The scope and sensitivity of testing met or exceeded current recommendations for impaired driving investigations and included relevant medicolegal death investigations compounds of interest.

S-011: A Forward-Thinking Approach to Tackling New Synthetic Opioid "Nitazene" Analogues by Liquid Chromatography Mass Spectrometry

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Background/Introduction: Synthetic opioids remain a constant challenge for forensic toxicologists as new drugs appear and turn over within a short timeframe. Beginning in 2019, benzimidazole analogues (known as "nitazenes") began appearing and proliferating within the United States. Isotonitazene was the first to be identified (2019), followed by metonitazene (2020), butonitazene (2021), flunitazene (2021), and etodesnitazene (2021). Increasing popularity of these drugs quickly demonstrated the need for sensitive, selective, and innovative methods for detection and quantitation.

Objectives: The objective of this study was to proactively develop an assay that would encompass both current and potential future "nitazene" analogues yet to be identified in forensic toxicology casework. This was achieved by developing and validating a quantitative confirmation method for nine "nitazene" analogues and metabolites using liquid chromatography tandem quadrupole mass spectrometry (LC-QQQ-MS). Ultimate utility of the validated method was assessed through application to authentic biological specimens.

Methods: "Nitazene" analogues included in this analytical method were isotonitazene, protonitazene, meto-nitazene, etonitazene, clonitazene, flunitazene, *N*-desethyl isotonitazene, 5-amino isotonitazene, and 4'-hydroxy nitazene. Isotonitazene-D7 was used as the internal standard. The calibration range was assessed from 0.5 to 50 ng/mL. Samples (0.5 mL) were prepared using a basic liquid-liquid extraction. Quantitation was performed using a Waters Xevo TQ-S micro LC-QQQ-MS (Milford, MA). Chromatographic separation was achieved on an Agilent InfinityLab Poroshell C-18 120 (2.7 µm, 3.0 x 100 mm) column using gradient elution. Mobile phase compositions were 0.1% formic acid in water and 0.1% formic acid in methanol. The flow rate was 0.4 mL/min and injection volume was 5 µL. Method validation was performed based on the guidelines set forth by the AAFS Standards Board. A stability study was added for all analytes. Authentic samples suspected to contain isotonitazene, metonitazene, and/or other new synthetic opioids were obtained for analysis from medical examiner and coroner offices, as well as other partnering forensic toxicology laboratories.

Results: The method was successfully validated for all "nitazene" analogues. Criteria were met or exceeded for calibration model, accuracy, precision, interferences, recovery, matrix effects, carryover, ion suppression, limit of detection, limit of quantitation, and processed sample stability. All analogues (except for 5-amino isotonitazene) passed dilution integrity and matrix matching studies, and were all stable in the refrigerator for at least 60 days. Isotonitazene was confirmed in 104 blood samples (collection time frame: October 2019 – January 2020), at concentrations ranging from <0.5 to 9 ng/mL, and its most prevalent metabolite (*N*-desethyl isotonitazene, n=101), ranged in concentration from <0.5 to 136 ng/mL. 5-amino isotonitazene (n=17) and 4'-hydroxy nitazene (n=67) were also confirmed in these samples. In addition to isotonitazene, several other "nitazene" analogues (e.g., metonitazene, flunitazene) were identified and confirmed after validation of the assay. Metonitazene was confirmed in 20 postmortem cases (collection time frame: November 2020 – February 2021) and flunitazene (n=3) was unexpectedly discovered and confirmed (February 2021). Butonitazene was discovered during the study, and the method was expanded to include this new drug, demonstrating the versatility of the assay. Metonitazene blood concentrations ranged <0.5 to 33 ng/mL, and the universal metabolite 4'-hydroxy nitazene was confirmed in the samples. Flunitazene was confirmed alongside metonitazene with blood concentrations ranging <0.5 – 2.1 ng/mL. Butonitazene was confirmed in one case (January 2021) at 3.2 ng/mL. The benzimidazole analogues confirmed using this method were commonly identified alongside NPS benzodiazepines, opioids, and adulterants (e.g., quinine).

Conclusion/Discussion: This assay identified "nitazene" analogues with appropriate sensitivity. Preemptive addition of unidentified analogues allowed for successful and timely characterization of new synthetic opioids as they quickly were introduced on the recreational drug market. This work provides a starting point for laboratories interested in pursuing innovative quantification methods for new benzimidazole ("nitazene") analogues.

PLATFORM

S-012: Analysis of Ethanol Aerosolized in an Electronic Cigarette by Headspace Gas Chromatography-Flame Ionization Detector (GC-FID) and Particle Size Analysis

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Background/Introduction: Electronic cigarettes (e-cigs) were designed to be a healthier alternative nicotine delivery system. E-cigs contain three main components: a battery-operated heating system, a coil/wicking system, and a cartridge or pod for the e-liquid. Generally, these e-liquids are composed of propylene glycol (PG), vegetable glycerin (VG), flavoring agents, and varying concentrations of nicotine. Ethanol has been found as an unlabeled ingredient in refill e-liquid formulations and non-refillable pods, ranging from 0 to >20% by volume. Ethanol is contained in e-liquids as a solvent for flavoring chemicals and drugs, as a thinning agent, and as a drug of abuse. The condensation aerosol formed by the e-cig deposits in different regions of the respiratory system. The most significant absorption occurs in the alveoli, which can potentially cause acute lung injury. Inhaled ethanol from vaping results in an average ethyl glucuronide concentration of 371 ± 43 ng/mL in urine.

Objectives: The purpose of this study is to quantify the amount of ethanol present in the condensation aerosol (n=10) from five e-liquids and to determine the size of the particles generated by a popular e-cig, called the SubOx Mini C, using a Nickel-Chromium (NiCr) 0.5 ohms resistance atomizer.

Methods: Capture of the aerosol was achieved by attaching the e-cig to a series of two chilled Buchner Flasks containing 150mL of deionized (DI) water each. The traps were attached to an electronic vacuum pump operating at 2.3 L/min. Each sample consisted of four 4-second aerosolization pulls. The tubing was rinsed with an additional 100 mL of DI water into the first flask and collected for further analysis. Five different e-liquids were created at set ethanol concentrations (0, 5, 10, 15, 20% [v/v]) and five samples were collected for each e-liquid. The samples (900 µL of sample, 100 µL of internal standard (ISTD) [0.234 mg/mL n-propanol]) were analyzed using a Shimadzu Nexis GC-2030 with HS-20 gas chromatography headspace sampler employing an isothermal method. Particle size analysis was achieved using a ten stage Micro-Orifice Uniform Deposit Impactor (MOUDI™) with samples consisting of ten 10-second pulls at 30 L/min. Each stage was lined with aluminum foil or filter paper, which was placed into a headspace vial with 1000 µL of the ISTD.

Results: The aerosols from the e-liquids (5, 10, 15, and 20% ethanol) yielded average ethanol doses of 907, 3032, 4772, and 6603 µg/puff and percent recoveries of 117, 170, 128, and 190% , respectively. Particle size results showed that on average over 80% of the aerosol fell within the mean mass aerodynamic diameter of 0.172-0.31 µm, with ethanol doses ranging from 141-1174 µg/puff.

Conclusion/Discussion: The average mean mass aerodynamic diameter for the aerosol particles falls within the range of sizes that are inhaled into the deep lung tissue and absorbed into the bloodstream. The inhaled dose in a typical "puff" is below what would typically be considered intoxicating. However, repeated micro-dosing of ethanol can lead to detectable concentrations of metabolites in urine, potentially precipitating failed drug tests. As a solvent, ethanol reaching the epithelial basement membrane at the interface of the alveoli and capillary beds can create oxidative stress leading to acute lung injury. As a consequence, the unlabeled ingredients of e-liquids may be a threat to public health and public safety.

PLATFORM

S-013: Quantification of Fentanyl Analogs in Oral Fluid using LC-QTOF-MS

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Background/Introduction: Fentanyl analogs continue to persist in the illicit drug community of the United States. In recent years, fentanyl analogs have not only been detected postmortem, but also in increasing numbers of antemortem casework such as, driving under the influence of drugs (DUID). Oral fluid is a viable matrix for antemortem investigations due to rapid, simple collection and indications of recent drug use. Limited research is available addressing fentanyl analog prevalence in oral fluid; therefore, development of detection and quantification methods for compounds involved in the opioid epidemic is critical.

Objective: The goal of this project was to validate a quantitative method for fentanyl analogs in oral fluid using liquid chromatography-quadrupole-time-of-flight mass spectrometry (LC-QTOF-MS) based on ANSI/ASB Standard 036. The method was then applied to authentic oral fluid samples collected from probationers/parolees.

Method: Analytes of interest were 3-methylfentanyl, 4-ANPP, 4-fluoroisobutyrylfentanyl, acetylfentanyl, acrylfentanyl, butyrylfentanyl, carfentanil, cyclopropylfentanyl, fentanyl, furanylfentanyl, methoxyacetylfentanyl, p-fluorofentanyl, and valerylfentanyl. Oral fluid samples (100 µL OF: 300 µL Quantisal® extraction buffer) were fortified with calibrators or quality controls (QCs), and internal standard (ISTD). Analytes were isolated using a previously published solid-phase extraction procedure utilizing co-polymeric mixed mode SPE cartridges and an elution solvent of 5% ammonium hydroxide in ethyl acetate. Analytes were separated and detected using an Agilent Technologies 1290 Infinity liquid chromatograph coupled to an Agilent Technologies 6530 Accurate Mass Time-of-Flight mass spectrometer (LC-QTOF-MS). Data were acquired in targeted acquisition mode. Quantification occurred by monitoring two ion transitions (quantifier and qualifier) for each analyte. The method was validated using ANSI/ASB Standard 036. Parameters assessed were linearity, precision and bias, limit of detection (LOD), lower limit of quantification (LLOQ), matrix effects, interferences, carryover, short-term-stability, and dilution integrity. For proof of applicability, authentic oral fluid samples (received from Redwood Toxicology) previously collected from probationers/parolees were analyzed (n=17).

Results: Established linear ranges were 1-100 ng/mL for all analytes, except acetylfentanyl with 0.5-100 ng/mL (R^2 >0.994). LOD were 0.5-1 ng/mL. Precision (within- and between-run) and bias were acceptable with maximum values of ±15.2% CV and ±14.1%, respectively, at three concentrations. Ion enhancement was observed at low (9.3-47.4%) and high (4.0-28.0%) concentrations. Although matrix effects exceeded ±25% for some analytes, ion enhancement observed in matched deuterated ISTDs were comparable. No interferences (endogenous, exogenous, or stable-isotope) or carryover were observed. All analytes were stable in processed extracts for 48h in the autosampler (4°C). Dilution integrity was sustained at a dilution factor of 1/2 (±15.5% bias). Authentic oral fluid samples were positive for fentanyl (n=16) and 4-ANPP (n=3) at concentration ranges of 1.0-104.5 ng/mL and 1.2- 5.7 ng/mL, respectively. Mean fentanyl and 4-ANPP concentrations were 32.9 and 3.4 ng/mL, respectively.

Conclusion/Discussion: This research presents a valid quantification method for fentanyl analogs in oral fluid by LC-QTOF-MS with a small sample volume and low detection limits. In addition, the method was successfully applied to the analysis of authentic oral fluid samples containing fentanyl and 4-ANPP. While limited fentanyl analogs were detected, the present method remains relevant for the evolving use of fentanyl analogs and the advancement of routine oral fluid analyses in forensic laboratories.

Keywords: Fentanyl analogs, Oral fluid, LC-QTOF-MS

PLATFORM

S-014: The Quantitation of Eutylone in Sexual Assault Cases using LC-MS/MS

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Background/Introduction: Drug-facilitated crimes (DFC) include drug-facilitated sexual assaults (DFSA), which can be defined as a sexual activity occurring whereby the victim is incapacitated by drugs and/or alcohol and thereby unable to consent. Urine is the most useful specimen in these cases due to the extended time window of drug detection, however blood samples give more information in respects to acute use and effects. According to the media, drugs commonly implicated in cases of sexual assault are flunitrazepam ("roofies"), gamma-hydroxybutyrate (GHB), and ketamine. That said, according to published literature, ethanol is the most frequently encountered drug in these victims, and any substance that can cause incapacitation through cognitive or psychomotor effects can render someone a victim of a DFSA. β-Keto-1,3-benzodioxolyl-N-ethylbutanamine is a synthetic cathinone, also known as eutylone, belonging to the phenethylamine class. Eutylone can cause a state of mental confusion, and when used in combination with other drugs can have a synergistic or additive effect. Side effects from eutylone use include euphoria, dizziness, vertigo, confusion, tachycardia, and agitation, which is like "molly" or "ecstasy," substances commonly considered to be Methylenedioxymethamphetamine (MDMA), which has been implicated in DFSA cases. This presentation will detail the validation of a quantitative stimulant panel in blood that includes eutylone, amphetamines, and other synthetic cathinones and the results of analyzed samples from victims of DFSA. DFSA cases analyzed were selected based on detection of eutylone in corresponding urine samples using qualitative GC-MS and/or LC-TOF screening panels.

Objectives: This project sought to validate a quantitative method for the detection of stimulant drugs including eutylone in blood and demonstrate its application to DFC cases.

Methods: 0.5 mL aliquots of blood fortified with an internal standard mix, containing eutylone-D5, were extracted using Cerex Trace-B solid-phase extraction (SPE) cartridges. Sample extracts were analyzed using an Agilent 1260 high performance liquid chromatography (HPLC) system coupled to an Agilent 6460 triple quadrupole mass spectrometer (MS/MS). Chromatographic separation was achieved using an Agilent Poroshell 120 EC (2.7 µM, 3.0 mm x 50 mm) analytical column. 2 µL of the extracted samples were injected using Jet-stream ESI mode and analyzed using dynamic reaction monitoring (MRM).

Results: Eutylone quantitation was validated following ASB 036 method validation standards. Eutylone had a limit of detection (LOD) of 1 ng/mL and a limit of quantitation (LOQ) of 5 ng/mL. Eutylone was linear from 5-2000 ng/mL using a $1/x^2$ weighting. Eutylone did not demonstrate significant ionization enhancement or suppression and demonstrated bias within ±10% and a %CV of 15% for intra- and inter-day precision. Since 2019, 10 DFSA cases submitted to the University of Miami Toxicology Laboratory confirmed eutylone in urine and evidence submission included a paired blood sample. Case demographics presented 90% female subjects ranging from 18 to 55 yrs. The average concentration was 107 ng/mL, with the highest eutylone concentration at 501 ng/mL. Three cases were below the LOQ.

Conclusion/Discussion: This robust method achieved low LOD and LOQ for eutylone. A wide range of eutylone concentrations were detected in multiple DFSA cases, including instances where no stimulant use was reported or suggested in the case history. From the results presented, testing for synthetic cathinones, should be incorporated into routine DFSA casework, even if not indicated by the victim. The side-effect profile of eutylone could lead a victim to be vulnerable and more susceptible as a victim of non-consensual sexual contact.

PLATFORM

S-015: Effective and Rapid Workflow for Blood and Urine Testing in Drug Facilitated Crimes Casework

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Background/Introduction: Drug Facilitated Crimes (DFC), including sexual assaults, continue to challenge forensic toxicologists as both volume and complexity of casework increase, and resources remain limited. Further, both SOFT and the AAFS Standards Board (ASB) have minimum limits of detection and scope of drugs and metabolites for urine analysis either established, or in the final stages of review. Increased drug detection sensitivity better allows for results to be obtained, even after an extended period of time has elapsed following suspected drug administration. Although analytical techniques continue to improve, laboratories often require multiple methods to screen, confirm, and quantitate blood and urine in poly-drug casework.

Objectives: To design a rapid, routine, and comprehensive multi-drug class screening and confirmation testing workflow for quantitative blood and qualitative urine analysis of 184 analytes applicable to DFC casework.

Methods: Two LC-MS/MS techniques were developed that each targeted a different set of drug classes (Method I and II, see Table 1). However, both methods utilized the same rapid extraction of 150 µL of blood or urine via protein precipitation followed by Thomson size exclusion filtration. Each separate extract was acquired on either an 8- or 5.5-minute run-time using the same Phenomenex Kinetex 2.6 µm 100 Å phenyl-hexyl 100 x 2.1 mm column. Mobile phase A consisted of 5 mM ammonium formate in deionized water and pH adjusted to 4.5 using formic acid; and, organic mobile phase B consisted of ACN with 0.1% formic acid (v/v). The LC-MS/MS system comprised of a Sciex Nexera X2 LC-30 coupled with a Sciex QTRAP 6500+ mass spectrometer utilizing an Ion Drive™ Turbo V electrospray ionization (ESI) source operating in positive and negative multiple reaction mode (MRM). Data processing was expedited with an in-house customized query that automated processing and enhanced quality assurance.

Results: Assessed quantitatively in blood and qualitatively in urine, 184 target drug and metabolite analytes were examined including benzodiazepines (36), sleep-aid Z-drugs (5), barbiturates (10), antihistamines (11), muscle relaxants (5), dissociatives and hallucinogens (8), anticonvulsants (9), antidepressants (20), antipsychotics (11), cannabinoids (12), amphetamines (11), cocaine (6), opioids (27), anesthetics (2), analgesics (2), alpha-adrenergics and cardiacs (3), GHB and precursor (2), and miscellaneous substances (2). Following validation to ASB/ANSI standards, applicability studies analyzed over 200 proficiency test and authentic DFC blood and urine samples to demonstrate effective detection of target analytes. Limits of detection are appropriate for DFC, meeting (and often exceeding) proposed ASB scope/sensitivity guidelines for DFC. Further, other forensic casework such as driving under the influence (DUID) and postmortem investigations may be analyzed, allowing this testing scheme to be utilized across multiple case types.

Conclusion/Discussion: By increasing the analytical scope of multiple drug classes via two methods performed in tandem with improved sensitivity, this testing scheme detects drugs that may have previously gone undetected. This streamlined workflow of just two LC-MS/MS methods significantly improves laboratory efficiency while mitigating bias and reducing false negatives by routinely analyzing all drugs and metabolites required for DFC casework.

S-016: The Road to a National Standard for the Scope and Sensitivity for Testing Urine in Investigations of Drug-Facilitated Crimes

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Background/Introduction: SOFT's *Recommended Minimum Performance Limits for Common DFC Drugs and Metabolites in Urine Samples* was used as a starting point for the OSAC/ASB *Standard for the Analytical Scope and Sensitivity of Forensic Toxicological Testing of Urine in Drug-Facilitated Crime Investigations.*

Objectives: This presentation will explain how the OSAC/ASB standard was created with the SOFT document as a starting point. Attendees will understand why the two documents are different, and how each can be used appropriately.

Methods and Results: In 2005, SOFT's Drug-Facilitated Crimes (DFC) Committee published its first version of the *Recommended Minimum Performance Limits for Common DFC Drugs and Metabolites in Urine Samples*. Over the last 16 years, the list has been twice updated. Its primary purpose is to serve as guidance for laboratories performing analyses of samples from alleged DFC victims. It is also intended to serve as a resource for forensic nurses, law enforcement, attorneys, and other users of forensic toxicology services. The list documents common drugs that may be encountered in cases in which drugs are suspected to have been used to facilitate a crime. It also defines detection limits that a laboratory should aim to reach in order to adequately rule out the presence of these drugs and metabolites in urine specimens collected up to 5 days after an incident. This document was meant to be a guidance document for laboratories to improve their testing capabilities and to ensure comprehensive, sensitive testing that may improve the likelihood of detecting drugs and metabolites several days after a single dose. In 2014, the National Institute of Standards and Technology established the Organization of Scientific Area Committees (OSAC) for Forensic Science to draft consensus standards for the forensic science community. The Forensic Toxicology Subcommittee developed three standards to define the minimum scope and sensitivity for various types of investigations, to include DFCs. Ownership of the OSAC's draft S*tandard for the Analytical Scope and Sensitivity of Forensic Toxicological Testing of Urine in Drug-Facilitated Crime Investigations* was passed to the AAFS Standards Board (ASB) so that it could undergo public comment with adjudication through an ANSI-approved consensus process. The document went through three rounds of public comment and comment adjudication, prior to its recent publication. The OSAC/ASB Scope and Sensitivity document delineates the minimum requirements for target analytes and analytical sensitivity for the forensic toxicological testing of urine specimens from alleged victims of DFC.

To draft the OSAC/ASB document, the authors started with the SOFT list. The drugs and metabolites included in the OSAC/ASB document were limited to those most likely to be encountered in a typical DFC case. Detection limits were reviewed to ensure they were achievable by toxicology laboratories using common instrumentation such as gas chromatography/mass spectrometry and liquid chromatography tandem mass spectrometry. As expected, the draft document was updated through the ASB's process. Changes were made pursuant to comments from the public, as well as from further discussion and evaluation by the ASB Toxicology Consensus Body. For example, concentrations of MDA and MDMA were lowered from 50 to 25 ng/mL, isomer discussion was removed, and consideration of regional drug trends and case history was changed from a requirement to a recommendation.

Conclusion/Discussion: SOFT's *Recommended Minimum Performance Limits for Common DFC Drugs and Metabolites in Urine Samples* was used as a starting point for the OSAC/ASB S*tandard for the Analytical Scope and Sensitivity of Forensic Toxicological Testing of Urine in Drug-Facilitated Crime Investigations.* The two documents differ in that SOFT's document is intended as guidance, while the OSAC/ASB document sets a minimum standard of practice for those laboratories performing toxicological analyses of cases involving allegations of DFC*.*

PLATFORM

S-017: Synthetic Cannabinoids in Sexual Assault Casework

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Background/Introduction: The recent focus on maintaining respiratory health has diverted smokers from tobacco use to e-cigarettes. These devices vaporize flavored liquids as a mechanism of inhaling compounds such as nicotine or cannabidiol without delivering the combustion products of smoke and tar, which are responsible for chronic lung disease. Although vaping liquids are marketed as a more desirable and safer alternative to cigarettes, they may contain a variety of adulterants, such as synthetic cannabinoids, which can cause extreme intoxication and hospitalization. The widening patterns of abuse now encompass drug-facilitated sexual assaults, where cannabinoid intoxication can lead to vulnerability and a loss of situational awareness.

Objectives: This presentation raises awareness on identifying and confirming prominent synthetic cannabinoid derivatives emerging in drug-facilitated sexual assault casework.

Methods: Blood and urine specimens were submitted to the toxicology laboratory as part of suspected drug-facilitated sexual assault investigations. Routine screening encompassed a drugs of abuse immunoassay for 9 drug classes and a volatiles screen by headspace gas chromatography with flame ionization detection. Additionally, a screen for gamma-hydroxybutyric acid by gas chromatography-mass spectrometry and a comprehensive liquid chromatography-quadrupole time of flight/mass spectrometry (LC-QTOF/MS) screen were used to fulfill the recommended scope of testing and limits of detection promulgated by the SOFT Drug Facilitated Crimes Committee. If the investigation indicated a history of vaping, a LC-QTOF/MS synthetic cannabinoid assay was also used to screen for a mix of parent/metabolite compounds which included modern indole or indazole-based cannabinoid agonists found in current casework. If the assay revealed any peaks that met acceptance criteria, a liquid chromatography/ tandem mass spectrometry (LC-MS/MS) confirmation assay was used to confirm the presence of cannabinoids at limits of detection ranging from 0.1 ng/mL to 1.0 ng/mL, based on the specific compound relative to the blood or urine matrix in which it was found.

Results: In the past 12 months, the laboratory received over 130 cases submitted for sexual assault investigations. As with most sexual assault laboratory results, the bulk of analyses returned with negative or unremarkable toxicology findings. Among those were 3 couples that noted a significant change in mental status after vaping together: Case 1 involved a female who was off-post with a male companion, where they visited several bars and consumed an unknown number of alcoholic beverages. Afterwards, they purchased a vape pen and shared it between them. The female reported feeling dizzy and disoriented. She had no memory of returning to quarters, but awoke to the male sexually assaulting her. Case 2 was a male and female sharing a hotel room, where they shared a vape pen and then reported feeling very strange. The female became paranoid and called police. The male was arrested but later released after determining no assault had occurred. Case 3 involved two males who were sharing a vape pen. They both felt "heavy", where one of them reported that an assault had occurred after the effects subsided. In each of these cases, the metabolite 5F-MDMB-PICA 3,3-dimethylbutanoic acid was confirmed.

Conclusion/Discussion: Vaping has proliferated among those who have turned away from tobacco use and among those who would likewise engage in the activity, since vaporized delivery is considered to be a more acceptable and safer experience than smoking cigarettes. This perception has led to a rapid and pervasive spread of synthetic cannabinoid intoxications that are a threat to health, safety, and readiness. Although most common in fit-for duty investigations, synthetic cannabinoids are now being observed in suspected cases of drug-facilitated sexual assault. It is recommended that laboratories have the capability to identify synthetic cannabinoid intoxications in sexual assault casework when necessary.

PLATFORM

S-018: Toxicological Analysis of Paired Blood and Urine Specimens in Drug Facilitated Crime Cases

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Background/Introduction: Drug facilitated crime (DFC) cases are a subset of human-performance toxicology cases that include drug-facilitated sexual assault (DFSA). Urine specimens are the routine specimens tested in these cases, as there may often be a delay between the incident and sample collection, and urine allows for a longer detection window after exposure. The Society of Forensic Toxicologists DFC committee has prepared minimum recommendations for target drugs and detection cutoffs in urine for DFC cases. Currently, the AAFS Standard Board is developing a standard to establish target compounds and cutoffs in urine to be required for DFC case testing. While these documents and many laboratories focus on testing urine specimens in DFC cases, paired blood specimens can also provide valuable information or support the victims' accounts. Although testing multiple specimens and matrices increases the total number of tests needed for each DFC case, laboratories with validated methods that employ the same instrumentation and target the same drug panels can reduce this burden.

Objectives: To examine paired analytical screening methods used to analyze urine and blood specimens and determine the significance of paired urine and blood testing in DFC cases.

Methods: DFC cases submitted to the University of Miami Toxicology Laboratory (UMTL) for forensic analysis between January 2020 and April 2021 were used as the case examples and calculated statistics. The analytical methods used to analyze specimens in DFC cases included a targeted liquid chromatography-tandem quadrupole mass spectrometry (LC-MS/MS) method for 80 drugs and metabolites in urine or 82 drugs and metabolites in blood. Additionally, comprehensive basic drug screens using LC-time-of-flight-mass spectrometry (LC-TOF) and gas chromatography-mass spectrometry (GC-MS) that can detect over 200 and 800 drugs and metabolites, respectively, were performed. All urine specimens were screened using the targeted LC-MS/MS screen and the non-targeted LC-TOF and GC-MS basic screens. If drugs and/or metabolites were detected in urine, blood specimens were subsequently analyzed using the paired method.

Results: From January 2020 to April 2021, 157 DFC cases were submitted to the UMTL; 147 (93%) of the DFC cases were submitted with blood and urine specimens. After screening the urine specimens in each case, 64% of the cases then had blood specimens analyzed using at least one of the listed methods and any additional confirmatory or quantitative methods, if applicable. The analysis of the blood specimens was significant in several of these cases. In one case, ketamine and norketamine were detected in the urine and were subsequently detected and quantified in the blood; this was significant because ketamine has a short half-life. The results indicated recent, not historical, exposure associated with the incident and were consistent with the victim's account. Other cases in which confirmation in blood was beneficial included a case in which eutylone was detected in urine and was the only drug confirmed in the blood, and a case in which alprazolam was detected in the urine and confirmed in the blood; both cases indicate recent exposure to impairing substances that could have contributed to the DFC.

Conclusion/Discussion: Although not applicable in all DFC cases due to specimen availability or delay in sample collection, analyzing both urine and blood specimens can impact DFC cases and aid in interpreting toxicological results. Blood specimen results provide additional information by corroborating a victim's statement or indicating a timeframe of exposure to drugs detected during analysis, as demonstrated in the case examples. Using similar analytical methods and techniques for urine and blood matrices allows for a faster and more efficient workflow while still testing for a broad range of drugs in both matrices; this can enable laboratories to routinely test paired urine and blood matrices in DFC cases.

PLATFORM

S-019: Examining the Evidence on Fluorofentanyl – Multidisciplinary Evaluation of this Emerging Drug with a Focus on Forensic Toxicology Investigations

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Background/Introduction: Around 2008, fentanyl began infiltrating the recreational heroin market in the United States (US), becoming dominant by the late 2010s. Today, fentanyl related deaths and incidental positivity continue to increase. Fentanyl maintains a strong hold on the recreational opioid market, fueled by illicit manufacture and distribution within North America. The recent emergence/re-emergence of fluorofentanyl raises the prospect of fentanyl being displaced as the primary illicit opioid in the US, at least potentially in some regions and/or drug supplies. Fluorofentanyl exists in three isomeric forms: *ortho*-, *meta*-, and *para*-fluorofentanyl.

Objectives: Fentanyl manufacture can be studied via the analysis of precursors and/or byproducts from clandestine synthesis (i.e., signature analysis or chemical fingerprint). Our laboratory (CFSRE) implemented sample-mining and data-mining approaches in 2018 to track emerging and evolving drug trends. The objective herein was to evaluate comprehensive toxicology data to determine positivity, prevalence, and important epidemiological factors with respect to fluorofentanyl. The goal of this presentation is to provide a multidisciplinary look at fluorofentanyl and how associated aspects (e.g., synthesis, proliferation, demographics) mimic fentanyl, but not other NPS.

Methods: Hundreds of biological samples, extracts, and seized drug materials from forensic and clinical toxicology investigations were obtained for comprehensive toxicology testing. Non-targeted acquisition using a SCIEX TripleTOF® 5600+ quadrupole time-of-flight mass spectrometer (LC-QTOF-MS) was employed. The library database included more than 900 traditional drugs, NPS, metabolites, and other relevant analytes. Confirmation of *ortho*- vs. *meta*- vs. *para*-fluorofentanyl was conducted using a Waters Xevo TQ-S micro tandem mass spectrometer (LC-QQQ-MS).

Results: Fluorofentanyl "re-emerged" in the recreational drug supply in late 2020 and continues to increase exponentially in positivity (of total cases: Q3 2020: 0%, Q4 2020: 0.4%, Q1 2021: 4.0% -- compared to fentanyl: Q3 2020: 58%, Q4 2020: 55%, Q1 2021: 55%). Fluorofentanyl is the first fentanyl analogue to gain traction in the US drug supply since the enactment of core structure scheduling of fentanyl related substances in 2018. Confirmatory analysis identified *para*-fluorofentanyl as the most commonly encountered isomer of this analogue. *para*-Fluorofentanyl was routinely found in combination with fentanyl. Concentrations of *para*-fluorofentanyl in blood ranged from less than 0.1 ng/mL to greater than 20 ng/mL. Analysis of drug materials showed varying ratios of *para*-fluorofentanyl to fentanyl (<0.1 to >25), and one counterfeit opioid tablet contained *para*-fluorofentanyl only. Signature analysis of both toxicology samples and drug materials uncovered the presence of byproducts related to fluorofentanyl (e.g., fluoro-4-ANPP, fluoro-phenethyl-4-ANPP). These byproducts matched those related to fentanyl from illicit synthetic routes. *In vitro* activity data for fluorofentanyl in the literature show the *para*- isomer to be similar in potency to fentanyl; however, the *ortho*- isomer is significantly more potent, stressing the need for differentiation during analytical testing. Cases involving fluorofentanyl originated from more than 30 states, spanning all regions of the US. This finding is dissimilar from other emerging synthetic opioids which were localized around the Midwest, before proliferating to other regions.

Conclusion/Discussion: The distribution of fluorofentanyl in forensic casework is increasingly similar to that of fentanyl in the US. Data show that fluorofentanyl is widespread in the opioid supply, both in combination with fentanyl and increasingly being found on its own. Byproducts detected in toxicology samples and drug materials suggest that the route of illicit manufacture is the same as that for fentanyl, but from different synthetic precursors. Unlike the recent emergence of NPS opioids (e.g., isotonitazene, brorphine), the appearance of fluorofentanyl seems to be linked to drug scheduling actions involving inactive precursors used to synthesize fentanyl (e.g., 4-AP) rather than the final drug itself. Overall, this trend may mark a new turning point in the illicit opioid landscape, which can have significant impacts on forensic toxicology practice.

PLATFORM

S-020: Phenethyl-4-ANPP: a marginally active byproduct suggesting a switch in illicit fentanyl synthesis routes

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Background/Introduction: The United States is suffering from a severe opioid crisis that has been largely driven by fentanyl. Extensive profiling of the illicit fentanyl supply is invaluable from surveillance and intelligence perspectives. An important strategy includes the study of chemical attribution signatures (e.g. trace amounts of synthesis precursors, byproducts/impurities in seized material, etc.). This information provides valuable insight into the employed synthesis routes at the heart of illicit fentanyl manufacture (previously, mainly the so-called Janssen or Siegfried methods), allowing to track and ultimately regulate crucial precursors. Recently, phenethyl-4-ANPP, a formerly unknown compound, was identified for the first time in seized fentanyl powder and biological samples. There are currently no reports on how this compound may have ended up in illicit drug preparations and whether its presence may have potential *in vivo* relevance.

Objectives: This presentation discusses different synthesis routes that may lead to the presence of phenethyl-4-ANPP in fentanyl preparations. Furthermore, we determined the *in vitro* µ-opioid receptor activation potential of phenethyl-4-ANPP to estimate its (ir) relevance *in vivo.*

Methods: Real-time and retrospective mass spectrometry (GC-MS/LC-QTOF-MS) data acquired from analysis of authentic forensic samples were used to monitor the illicit fentanyl supply. Data were acquired in a non-targeted approach and interrogated using sample-/data-mining. The *in vitro* µ-opioid receptor (MOR) activation potential of phenethyl-4-ANPP was evaluated via a cell-based NanoBiT® assay: activation of MOR, fused to one part of a nanoluciferase enzyme, leads to recruitment of β-arrestin2, fused to the other part of the enzyme. The resulting functional complementation restores the nanoluciferase activity, yielding a bioluminescent signal upon addition of the substrate furimazine.

Results: Phenethyl-4-ANPP was first identified in a fentanyl powder sample seized in April 2019, followed by its identification in a biological sample in December 2019. Between 2019-Q4 and 2020-Q3, phenethyl-4-ANPP was detected in 25/1054 fentanyl cases in the US. Three possible fentanyl synthesis routes are presented which, when badly executed in a single reaction vessel, may involve the formation of phenethyl-4-ANPP. A reaction that combines 4-anilinopiperidine with excess phenylethyl halide is most likely employed. The extent of MOR activation caused by 100 µM phenethyl-4-ANPP is comparable to that exerted by a roughly 100,000-fold lower concentration of fentanyl (0.001 µM-0.336 ng/mL). Hence, given the limited *in vitro* MOR activation potential, *in vivo* concentrations of phenethyl-4-ANPP are not expected to result in relevant opioid activity.

Conclusion/Discussion: Phenethyl-4-ANPP was recently identified for the first time in seized fentanyl powder samples and biological samples. We hypothesize that its presence is the result of a shift in fentanyl synthesis routes in an attempt to circumvent restrictions on previously used precursors. Negligible *in vitro* opioid activity, combined with its low abundance in fentanyl preparations, most likely renders phenethyl-4-ANPP biologically irrelevant *in vivo*. As clandestine operations are constantly changing shape, monitoring of fentanyl attributions continues to play an important role in our understanding and control of the illicit fentanyl supply.

PLATFORM

S-021: *In vitro* functional characterization of a panel of non-fentanyl opioid new psychoactive substances

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Background/Introduction: The landscape of new psychoactive substances (NPS) is constantly evolving, with new compounds entering the illicit drug market at a continuous pace. Of these, NPS with opioid effects form a particular threat owing to their high potency and prevalence. Whereas previously, the use of fentanyl and fentanyl derivatives was the main point of attention, legislations have reacted accordingly, which may have been a driving force towards the (ab)use of alternative μ-opioid receptor (MOR) agonists. In contrast to fentanyl and fentanyl analogues, details on these novel non-fentanyl opioid NPS are scarce. However, the increasing amount of case reports on (sometimes fatal) intoxications involving these drugs stresses the danger of this knowledge gap.

Objectives: The aim of this work was to profile a diverse set of recent non-fentanyl opioids in terms of their MOR activation potential. Such pharmacological profiling may eventually help direct and prioritize scheduling and harm reduction strategies.

Methods: We investigated the MOR activation potential of a large panel of recent MOR agonists, including several cinnamylpiperazines (2-methyl AP-237, *para*-methyl AP-237, AP-237, AP-238), U-compounds (2,4-difluoro-U-48800, 3,4-difluoro-U-47700, bromadoline/U-47931E, *N*-ethyl-U-47700, spiradoline/U-62066) and other structurally diverse opioids (2-fluoro viminol, bromadol, brorphine, butorphanol, isotonitazene, ketobemidone, mitragynine, 7-OH-mitragynine, MT-45, piperidylthiambutene, tianeptine). Two previously reported, closely related *in vitro* MOR activation assays were used to monitor G protein (mini-$G_{\alpha i}$) or β-arrestin 2 (βarr2) recruitment. The assays are based on the functional complementation of a split nanoluciferase (NanoLuc Binary Technology®, Promega): activation of MOR, fused to one part of the nanoluciferase, leads to recruitment of either βarr2 or mini-$G_{\alpha i}$, fused to the other part. This results in a restoration of the enzymatic activity, producing a measurable bioluminescent signal upon addition of a substrate. This set-up also allowed the assessment of biased agonism via previously published equations with hydromorphone as an unbiased reference compound.

Results: MOR activation profiles were obtained for all tested compounds, with values for potency (EC_{50}) ranging from the low nM to μM range. Among the evaluated cinnamylpiperazines, AP-238 was the most potent analogue ($EC_{50,\beta arr2}$ = 248 nM), whereas *N*-ethyl-U-47700 was the most potent U-compound of this set ($EC_{50,\beta arr2}$ = 451 nM). Bromadol, brorphine, isotonitazene, ketobemidone, *N*-ethyl-U-47700, piperidylthiambutene and tianeptine were among the opioids with the highest efficacy (Emax) values, exceeding that of the reference compound hydromorphone ≥1.3-fold (βarr2 assay) and >2.5-fold (mini-$G_{\alpha i}$ assay). The employed assays identified several partial agonists compared to hydromorphone, including 2-fluoro viminol ($E_{max,\beta arr2}$ = 21.2%), bromadoline/U-47931E ($E_{max,\beta arr2}$ = 52.8%), butorphanol ($E_{max,\beta arr2}$ = 43.8%), mitragynine ($E_{max,\beta arr2}$ = 19.2%) and 7-OH-mitragynine ($E_{max,\beta arr2}$ = 43.5%). No statistically significant bias was found for any of the tested MOR agonists.

Conclusion/Discussion: We systematically investigated the *in vitro* MOR activation potential of a diverse panel of recent non-fentanyl opioid NPS. Pharmacological profiling of such newly emerging substances is crucial to make a realistic estimation of the potential danger their use might bring along. This, in turn, may allow to prioritize legislative efforts towards controlling (variants of) these emerging drugs. Given the high potencies and efficacies of many compounds in the studied panel, intensive monitoring and proactive control measures remain of paramount importance.

PLATFORM

S-022: NPS Discovery: Evolution of Novel Synthetic Opioids in the United States with a Focus on Metonitazene

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Background/Introduction: Following the decline in prevalence of esoteric fentanyl analogs beginning in 2019, there has been a steady stream of novel synthetic opioids (NSO) that have made forensic toxicological analyses more complex. These new drugs appear frequently, often as part of a series, are encountered in overdoses and deaths typically for a period of months to less than a year and are then succeeded by a new illicit drug. Having a means of rapidly identifying and characterizing these newly emergent compounds and documenting their involvement in deaths and overdoses, can accelerate the process of collecting epidemiological data, adding the drugs to routine analytical scopes, detecting additional drug-caused and drug-related deaths, and assisting with getting the drugs scheduled. The NPS Discovery Initiative was developed based on a five-step process of intelligence gathering, surveillance, monitoring, response, and forecasting, to anticipate and document the discovery, appearance, proliferation and decline of NSO's in drug caused deaths, and to maintain currency with the next emerging compounds.

Objectives: This presentation looks at the sequence of identifications of NSO's from the NPS Discovery program, focusing on the progression from fentanyl analogs and cyclohexyl-N-methylbenzamides (U-series) in 2018, to cinnamyl piperazines (AP-series) and benzimidazoles (nitazenes) in 2019, including the most prominent new member of that series, metonitazene. We have previously reported on the life-cycle of isotonitazene, a novel benzimidazole NSO and the first in that series to enter the US drug supply in recent years.

Methods: This presentation will include a description of the use of the CFSRE NPS Discovery approach to track the proliferation and decline of metonitazene, which succeeded isotonitazene beginning in Q4 of 2020.

Results: Metonitazene was identified and quantified in 21 blood samples from postmortem casework, one serum sample, 15 urine samples, and 11 vitreous samples. The majority of cases were determined to be multiple drug toxicity deaths, and the manner was typically accident. The mean concentration of metonitazene in blood was 6.3±7.5 ng/mL (median: 3.8 ng/mL, range: <0.5-33 ng/mL). The blood concentrations of metonitazene were slightly higher than the concentrations previously encountered for isotonitazene and brorphine encountered in similar case circumstances, and metonitazene is believed to have potency similar to that of fentanyl. The majority of metonitazene cases were collected and submitted for analysis between January and February 2021; however, the earliest case was collected in November 2020. Eighteen (90%) decedents were male and two were female. The mean age was 41±12 years (median: 42 years) and ranged from 19 to 63 years. Cases were submitted from seven states, mostly centered around the Midwestern U.S. The majority of cases were from Illinois (n=5, 25%) and Tennessee (n=10, 50%). The remaining states included one case each from Iowa, Wisconsin, South Carolina, Florida, and Ohio. Metonitazene was the sole drug of interest in only three cases (15%), and was the only opioid detected in only six (30%) cases. Metonitazene was commonly found in combination with additional opioids (e.g., fentanyl), other NPS (e.g., benzodiazepines), and traditional stimulants (e.g., methamphetamine). NPS benzodiazepines were the most common subclass found in combination with metonitazene, including clonazolam (n=4), etizolam (n=2), flualprazolam (n=2), and pyrazolam (n=1).

Conclusion/Discussion: As with all emerging NPS drugs, laboratories should consider these results in the context of including emerging drugs in testing panels, and toxicologists should consider these results when cases of apparent opioid related death test negative for legacy or well characterized NSO's, and when interpreting casework results involving metonitazene, or current and future analogues.

PLATFORM

S-023: Emergence of the Novel Opioid, Metonitazene, in Postmortem Toxicology and Detection by LC-QToF-MS and LC-MS/MS

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Introduction: After the United States (US) Federal government used their emergency scheduling powers to schedule "fentanyl-related substances" in 2018-2019, non-fentanyl-related opioids began to emerge on the illicit drug market. Isotonitazene was the first of the nitazene family of compounds reported in the US in November 2019 and made a controlled substance in August 2020. A structurally related compound, metonitazene, was first reported in July 2020, but was not detected in our casework until November 2020. Metonitazene is not chemically similar to other opioids such as the morphinans (diacetylmorphine, morphine, etc.), the fentanils (fentanyl, carfentanil, etc.), or the U-family of compounds (U-47700, U-50488, etc.). During early pharmacological testing, metonitazene was determined to have approximately ten to one hundred times the analgesic potency of morphine.

Objectives: We report two of the earliest detections of metonitazene by the forensic toxicology laboratory – both of which occurred in Butler County, Ohio during November and December 2020. Case 1 involved a 42 year old male who was found unresponsive in a locked bathroom with a syringe. After resuscitative attempts, he was pronounced deceased. Case 2 involved a 43 year old male who was found lying in the bathroom doorway of his home after conversing with a family member. He was pronounced deceased at the scene.

Methods: Blood and urine specimens for toxicological analyses were obtained by the coroner. Blood was collected in tubes containing the additive sodium fluoride. Initial blood testing included a screen for drug and metabolites by liquid chromatography quadrupole time of flight mass spectrometry (LC-QToF-MS). Volatiles were tested by headspace gas chromatography with flame ionization detection (HS-GC-FID). Confirmatory analyses for all compounds in blood were completed by liquid chromatography with triple quadrupole mass spectrometry (LC-MS/MS). Urine was analyzed for drugs and metabolites by LC-MS/MS. All analytical methods were validated according to in-house validation standard operating procedures.

Results: *Case 1:* No autopsy was performed. The femoral blood specimen was positive for metonitazene (qualitative), methamphetamine (1,321 ng/mL), amphetamine (76.2 ng/mL), fentanyl (5.9 ng/mL), norfentanyl (2.5 ng/mL), delorazepam (20 ng/mL), quinine (380 ng/mL), and naloxone (qualitative). The urine specimen was positive (qualitative) for the following: methamphetamine, amphetamine, lorazepam, fentanyl, norfentanyl, and morphine. Cause of death was certified by the coroner as multiple drug toxicity including methamphetamine, fentanyl, and delorazepam. Manner of death was accident. *Case 2:* No autopsy was performed. The femoral blood specimen was positive for metonitazene (qualitative), fentanyl (5.3 ng/mL), norfentanyl (0.3 ng/mL), alprazolam (24.8 ng/mL), delta-9-THC (4.6 ng/mL), THC-carboxylic acid (56.0 ng/mL), gabapentin (0.7 mcg/mL), sertraline (279 ng/mL), desmethylsertraline (1,242 ng/mL), and cotinine (qualitative). The urine specimen was positive (qualitative) for methamphetamine, alprazolam, alpha-hydroxyalprazolam, fentanyl, and norfentanyl. Cause of death was certified by the coroner as multiple drug toxicity including fentanyl. Manner of death was accident.

Conclusion: Metonitazene emerged as a novel opioid in forensic toxicology in 2020. The analytical methods presented proved to be accurate and reliable for the detection and identification of metonitazene in blood specimens. We detected metonitazene in two postmortem blood cases. The detection of metonitazene in these cases was not considered relevant in the certified cause of death, but with the scarcity of available published literature specifically on this compound, we believe these case reports to be a good addition to existing reports surrounding the emergence of the nitazene family of compounds in postmortem toxicology.

S-024: Case Series Involving Novel Cinnamylpiperazine Synthetic Opioids: 2-Methyl AP-237 and AP-238

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Background/Introduction: AP-237 (bucinnazine) is a cinnamylpiperazine opioid that is used therapeutically in China for the treatment of cancer related pain but is not approved for use in the United States. Two analogues of this drug, 2-methyl AP-237 and AP-238, recently emerged in the recreational drug supply in 2019 and 2020, respectively. 2-Methyl AP-237 first appeared in a patent in Italy in 1983 and showed similar potency and toxicity to AP-237. No pharmacological data has been reported for AP-238. Unpublished data suggest this series of opioids has potency similar to that of morphine.

Objectives: The objective of this presentation is to discuss analytical findings of cinnamylpiperazines, 2-methyl AP-237 and AP-238, in authentic specimens in conjunction with case histories and cause and manner of death.

Methods: Analysis was performed using a Waters Xevo TQ-S Micro tandem mass spectrometer coupled with a Waters Acquity UPLC® (Milford, MA). Chromatographic separation was achieved using an Agilent Poroshell EC C-18 column (3.0 mm x 100 mm, 2.7 um) (Santa Clara, CA) heated to 30°C with a flow rate of 0.4 mL/min. The mobile phases were 0.1% formic acid in water and 0.1% formic acid in methanol. Biological specimens (0.5 mL) were extracted using a basic liquid-liquid extraction (pH=10.4). Quantitation of 2-methyl AP-237 and AP-238 was achieved using standard addition. Samples were diluted, as necessary, to fit into this range based on previous analysis.

Results: Samples from six forensic postmortem cases and one clinical case that either screened positive or scene findings indicated the use of 2-methyl-AP-237 (n=5) or AP-238 (n=2) were submitted for quantitative analysis. The cases were received between February 2020 and April 2021. All cases were from different geographical locations: California, Georgia, Tennessee, New Jersey, Floida, Illinois, and Texas. Demographic information was available for all but one case. The average age (±SD) was 32(±5) years. All individuals were male. The mean (±SD) and median concentrations of 2-methyl-AP-237 in blood were 1828 ng/mL (±2279) and 1100 ng/mL, respectively, with a range of 21-5800 ng/mL. Three matrices (e.g., blood, urine and vitreous) were received for one case with resulting concentrations of 1100, 5000, and 270 ng/mL, respectively. Other significant toxicological findings included naloxone (n=4) and novel benzodiazepines (n=2). The clinical case involving 2-methyl-AP-237 involved a 31-year-old male found unresponsive at home. Upon admission to the emergency department, he had the following vital signs: HR 65, BP 122/71, RR 18, and temperature 97.9°C. The individual was treated by emergency medical services with naloxone (0.4 and 0.8 mg IV) followed by ED administration (0.4 mg/hr IV), resulting in improvement in respiratory and mental status. Twenty-four hours after use, all opioid intoxication symptoms resolved. Upon further discussion, the individual reported using 1 gram of "2-methyl-AP-237" dissolved in 30 mL of water through a nasal spray bottle, as well as novel benzodiazepines (e.g., etizolam, and pyrazolam). One case involving AP-238 was associated with a blood concentration of 87 ng/mL and a urine concentration of 120 ng/mL. A second case had a blood concentration of 270 ng/mL and a urine concentration of 1200 ng/mL. Both cases additionally tested positive for novel benzodiazepines. The causes of death for both cases were listed as AP-238 toxicity.

Conclusion/Discussion: The majority of case histories involving 2-methyl AP-237 and AP-238 indicated the purchase of these drugs on the "dark web/black market", or the desire to obtain a synthetic heroin. This data shows that opioid overdoses are no longer just fentanyl-related compounds, and that it is important to make sure methodologies in forensic laboratories are monitoring for various non-fentanyl opioids. To our knowledge, these are the first reported deaths involving 2-methyl AP-237 and AP-238 in the literature, including the first reported drug concentrations.

PLATFORM

S-025: The United Nations Office of Drugs and Crime's (UNODC) Early Warning Advisory Toxicology Portal and Expanding Your Laboratory's Scope of Analysis for Novel Psychoactive Substances.

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Background/Introduction: The United Nations Office of Drugs and Crime (UNDOC) has established an Early Warning Advisory Toxicology Portal (EWA Tox-Portal) for novel psychoactive substances. Laboratories are encouraged to submit data on a quarterly basis, which is then compiled into a report and distributed to the participants. As the EWA Tox Portal can be accessed at any time, a laboratory can view the charts of drug information submitted, filter by country and/or analyte(s), or consult the quarterly report compiled by the UNODC. The Alabama Department of Forensic Sciences has contributed data since 2018 and is currently utilizing the EWA Tox-Portal to determine which stimulants and hallucinogens to add to the scope of analysis.

Objectives: To illustrate the use of the UNODC's EWA Tox-Portal by a forensic toxicology laboratory in order to expand their scope of analysis for novel psychoactive substances.

Methods: The Alabama Department of Forensic Sciences (ADFS) routinely submits cases to the UNODC's EWA Tox-Portal. Each quarter the UNODC will email participants requesting data for novel psychoactive substances which have been detected by the laboratory during that time period. This information includes novel psychoactive substances detected, analytical method(s) used, type of event, and the location of the laboratory. Cases can be entered into the portal individually or on a spreadsheet supplied by the UNODC if there is a large number of entries.

Results: In 2020 ADFS contributed to the EWA Tox-Portal by submitting 73 cases involving designer benzodiazepines, with some having multiple analytes present. The data from the EWA Tox-Portal shows an increase in the frequency of designer benzodiazepines appearing in various types of toxicology cases. In 2020, 73% of the top 10 most reported substances worldwide were benzodiazepines, while in 2019 benzodiazepines only accounted for 45%. Flualprazolam was the most common substance reported in both 2020 and 2019 but was not among the most common substances in 2018. Of all drugs listed in the EWA Tox-Portal worldwide, 50% are novel benzodiazepines and 35% of those were reported from the United States. Currently, flualprazolam is the most prevalent substance submitted to the EWA Tox-Portal both globally and in the United States with 721 and 550 listings, respectively. Flubromazolam was the third most submitted substance globally and the fourth most submitted from the United States. Prevalence among benzodiazepine data submitted to the EWA Tox-Portal by ADFS in 2020 showed trends similar to the United States in general and the current benzodiazepine assay did not need to be adjusted. The Alabama Department of Forensic Sciences has identified novel stimulants and hallucinogens within the EWA Tox-Portal to add to current validations for 2021. Both ketamine and Kratom have been in the global top 10 most reported substances since 2018. The limited data for early 2021 lists 3-methylmethcathinone and 3-methoxyphencyclidine in the top 10 substances. Regarding the most reported substances in fatality cases for early 2021, 2-fluorodeschloroketamine, 5-Methoxy-N,N-dimethyltryptamine, and 3-Methoxyeticyclidine have been identified.

Conclusion/Discussion: Due to the increased use of novel psychoactive substances and the rate at which the popularity of use changes from one drug to another, toxicology laboratories may not detect some of the newer substances. By utilizing the information contained within the UNDOC's EWA Tox-Portal, a laboratory can prioritize these substances for validation. Also, EWA Tox-Portal contributors are encouraged to include case histories to characterize expected effects and behavior observed with new drugs. The EWA Tox-Portal can be a very important tool in raising awareness of emerging novel psychoactive substances.

S-026: Designer Benzodiazepines -*In Vitro* Experiments and Toxicological Interpretation of Authentic Forensic Investigations

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Background: The detection of designer benzodiazepines (DBZDs) in forensic casework started in 2007 with the emergence of phenazepam. Subsequent to the detection of etizolam as a DBZD in 2011, several DBZDs started to be offered online. Flubromazepam, flubromazolam, clonazolam, and most recently flualprazolam, are still detected in casework. For DBDZs, the lack of experimental data imposes limitations when interpreting toxicological results. Furthermore, potential chemical instability might lead with false negatives. This has occurred in pharmaceutical nitro-BDZs such as clonazepam or flunitrazepam, where the 7-amino counterpart is not only a metabolic product, but also a product of the spontaneous reduction of the nitro group observed in post-mortem (PM) blood samples and in aqueous solutions.

Objectives: The aim of this study was to report quantitative data of two prevalent DBDZs, flubromazolam and clonazolam, confirmed in post-mortem (PM) and Driving Under the Influence (DUID) blood samples submitted to a large reference laboratory in 2020. Additionally, to identify clonazolam analytical targets by *in vitro* metabolism studies.

Methods: Blood samples from PM and DUID cases were quantified for clonazolam and flubromazolam via a quantitative LC-MS/MS routine assay after positive detection via routine screening assays. Additionally, 8-aminoclonazolam presumptive positives indicated in a LC time-of-flight (TOF)-MS screening method were considered to compare with those clonazolam positive cases. Confirmation of 8-aminoclonazolam cases were performed as special investigation via a standard addition LC-MS/MS method. Clonazolam was incubated with pooled human liver microsomes (pHLM) and recombinant CYP-enzymes. Metabolite identification was performed using liquid chromatography high resolution mass spectrometry (LC-HRMS, Q Exactive).

Results: Flubromazolam was confirmed in 196 blood samples in 2020, while clonazolam was confirmed in 43 blood samples (Figure I). However, using 8-aminoclonazolam as a biomarker of intake increased the positivity to 101.

Table I	Peripheral blood concentration (ng/mL)			
	Median	**Mean**	**Range**	**N**
Clonazolam	8.1	11	5.0–86	43
8-Aminoclonazolam	64	117	9.9–570	16
Flubromazolam	12	28	2.8–760	115
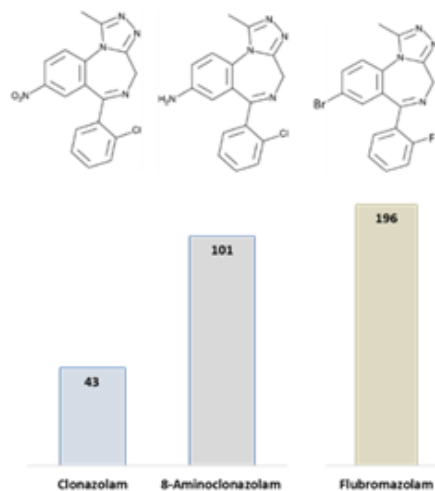
Figure I. Confirmations of clonazolam and flubromazolam in 2020, and presumptive 8-aminoclonazolam positives

Within the tested samples, flubromazolam was highly prevalent in Pennsylvania (16% of total samples positive for this analyte) and Tennessee (12%), followed by New York (7%) and Nevada (6%). For 8-aminoclonazolam, 23% of positives were from samples submitted by agencies in Illinois, 12% from South Carolina, 7% Tennessee and 6% Florida. On the other hand, 54% clonazolam positivity was from samples submitted in Pennsylvania and 9% Washington.

Discussion: The clonazolam positive rate was 3% out of 2206 blood samples positive for at least one DBDZs in 2020; however, 8-aminoclonazolam monitoring increased the clonazolam detection window. In cases where 8-aminoclonazolam was confirmed (n=16), the resultant concentrations were higher than those for clonazolam (Table I). Incubations of clonazolam with pHLM over 3 h showed a low hepatic elimination rate mostly mediated by CYP3A4, where the major metabolite identified was 8-aminoclonazolam. It suggested that the high positivity of 8-aminoclonazolam compared to clonazolam might be also due to stability issues. As observed for clonazepam, high 8-aminoclonazolam-clonazolam ratios in PM blood samples might also be a result of bacterial contamination in containers without preservatives. This is currently under investigation.

Conclusion: Quantitative and demographic data of two prevalent DBDZs and 8-aminoclonazolam was presented. Metabolic studies and 8-aminoclonazolam monitoring in forensic investigations supported that the major contribution of 8-aminoclonazolam might be likely due to stability issues in biological specimens, which is currently under investigation. Monitoring 8-aminocloanzolam seemed highly relevant in casework not only as an indicator of potential clonazolam intake, but also might provide a more accurate demographic information of this DBDZ.

S-027: Drug Recognition Expert (DRE) Evaluations Involving Synthetic Cannabinoids

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Background/Introduction: Drug recognition experts (DREs) are police officers trained to recognize impairment related to drugs other than alcohol. Since 2010, the market has been saturated with novel psychoactive substances (NPS) complicating DRE evaluations. The synthetic cannabinoid subclass of NPS has persisted with a constant influx of new analytes and structural isomers of classic synthetic cannabinoids. While of one of the seven DRE categories includes cannabis, there is limited research on the symptomology or effects related to synthetic cannabinoids within the context of the DRE evaluation. Reports in the literature are primarily focused on older generation synthetic cannabinoids that are no longer detected with any frequency. There are very limited impaired driving case reports on newer synthetic cannabinoids such as 5F-MDMB-PICA and MDMB-4en-PINACA. Further, few case reports exist where only a single drug or drug class is toxicologically confirmed.

Objectives: The objective of this presentation is to describe the effects of synthetic cannabinoids on DRE evaluations, in cases positive for synthetic cannabinoids in the absence of other drug findings.

Methods: Participation in the evaluation was voluntary and administered as part of a DRE certification program. Subjects were assessed by the 12-step standardized evaluation protocol for drug influence, which included eye examination, Standardized Field Sobriety examination, and clinical measurements. At the conclusion of the evaluation, an oral fluid sample was collected using the Immunalysis Quantisal™ device for laboratory-based analysis. Samples were analyzed using a Sciex TripleTOF® 5600+ quadrupole time-of-flight mass spectrometer coupled to a Shimadzu Nexera ultra high-performance liquid chromatograph, with synthetic cannabinoid cutoffs ranging from 0.1-1 ng/mL.

Results: Oral fluid specimens were positive for only synthetic cannabinoids in 13 of the subjects evaluated by a DRE. Seven samples were positive for 5F-MDMB-PICA, three for MDMB-4en-PINACA, one for ADB-BINACA and one containing both MDMB-4en-PINACA and ADB-BINACA. In all but one sample with only 5F-MDMB-PICA (n=7), an indole, no nystagmus was noted. LOC was noted in two 5F-MDMB-PICA cases. Comparatively, in cases positive for MDMB-4en-PINACA and/or ADB-BINACA, both indazoles, nystagmus was noted in four of the six cases with an angle of onset at or prior to 45° in three cases, which is not present with cannabis use. Three of these cases also had LOC. Pupil sizes were within normal ranges for all three light conditions for all but one participant. In all 13 evaluations, clues were noted by the DRE for the WAT test (e.g., missing all heel-to-toe steps, inability to keep balance, stepping off the line) and OLS test (e.g., swaying while balancing, using their arms, hopping and putting their foot down). Irrespective of the synthetic cannabinoid detected, sway (1 to 2 inches) was noted in all subjects. Muscle tone varied among the participants. Of the 10 cases where an opinion was available, only three opinions included cannabis.

Conclusion/Discussion: DRE opinions are rendered based on physiological and behavioral indicators consistent with specific drug categories. NPS, such as synthetic cannabinoids, have added complexity to the DRE evaluation. While synthetic cannabinoids and traditional cannabinoids bind to the same endogenous cannabinoid receptors, the presentation of effects, both physiological and behavioral, are not consistent with botanical cannabis. Although there are a limited number of cases presented here, there was some differential physiological effects observed, which appear to be related to the synthetic cannabinoids' core structure and further research is warranted. Due the persistence and constant evolution of synthetic cannabinoids and their potential to cause driving impairment, comprehensive toxicology is strongly recommended, even in cases where cannabinoids may not be suspected or opined by the DRE.

PLATFORM

S-028: The Effects of a Global Pandemic on Impaired Driving Trends in Southern Nevada

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Background/Introduction: During the COVID-19 global pandemic that spanned from March 2020 – April 2021 many changes were seen in daily life. For the first time in modern history the Las Vegas Strip was completely closed down and stayed dark and empty until June 2020. There were also restrictions on students going to school, people going to work, social gatherings and social events that have been a part of our daily lives for many years. This presentation will review the trends seen in impaired driving cases analyzed by the Henderson Forensic Laboratory during this time period and how they compared with impaired driving trends from previous years. The presentation will look at how unprecedented daily social changes such as long-term quarantine isolation from work, distance learning from home for children, changes in social patterns and overall "lockdown lifestyle" may have had on the impaired driving drug trends seen by the Henderson Forensic Laboratory.

Objectives: The objective was to analyze previous years DUI trends and data to see how they compared to what was seen during the COVID-19-time frame and encourage the audience to review the data to see if there were similar impaired driving trends in the areas that they work and live.

Methods: Whole blood samples from suspected impaired drivers were collected and analyzed for both alcohol and drugs, regardless of the blood alcohol concentration. This policy has been in place for the past 4 years, so previous years' data had similar parameters in reference to the depth of toxicology testing for impaired driving cases. Samples were screened via LC/Ion Trap for 98 different compounds using a modified QuEChERS extraction and any positive screening results were sent on for further confirmation and quantitation testing via LC/MS/MS using a modified liquid-liquid protein precipitation extraction.

Results: In reference to COVID-19 concerns, Henderson Police Department general order 20-002 was authorized, eliminating the use of breathalyzers to arrest suspects in impaired driving cases. This caused a 52% increase in the number of cases (593 to 902) compared to previous years due to all suspected impaired driving cases requiring a blood draw and laboratory analysis. Even with the substantial increase in the number of cases, the percentage of cases that were positive for drugs stayed in line with what was seen during previous years. As the COVID-19 restrictions initially implemented in March and April extended into the summer and fall seasons there was a notable increase in the number of drugs seen in each drug positive case. The following details the average number of drugs that were reported in cases where drugs were detected in the sample:

March 2019 – March 2020 = 2.4 drugs/case
April 2020 – July 2020 = 2.8 drugs/case
August 2020 – December 2020 = 3.2 drugs/case
January 2021 – April 2021 = 4.2 drugs/case

There was a notable increase in the number of benzodiazepine and synthetic benzodiazepine cases seen during this time. In the previous two years the Henderson Forensic Laboratory had seen a 13% decrease in the number of benzodiazepine cases.

Conclusion/Discussion: Immediate changes to daily lifestyles and transition to the new "normal" caused a lot of stress and anxiety in the daily lives of many people. This will be studied and analyzed for years to come to come by many branches of physical and social science research groups. It seems to also have affected the DUI trends that were seen at the Henderson Forensic Laboratory. As more areas of the country are starting to open back up again, it will be interesting to see how this return to "normal" social and lifestyle habits affect DUI trends in the years to come.

S-029: Popular Drug Combinations in DUID Investigations 2018-2020

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Background/Introduction: Drug impaired driving continues to pose a public safety risk. Thomas et. al. documented increases in single drug and polydrug positivity among seriously and fatally injured drivers on the road between 09/10/19 and 07/18/20 of 14% and 8%, respectively (1). Given the enhanced effects of certain drug combinations, the rise in polydrug use is a concerning trend.

Objectives: This presentation will review the prevalence of polydrug use in DUID cases submitted to NMS Labs between 2018 and 2020. Popular drug combinations will be presented with several case studies.

Methods: A total of 56,251 blood specimens were submitted to NMS Labs from across the United States for routine DUID testing between 2018 and 2020. This panel includes an Enzyme Linked Immunosorbent Assay (ELISA) screen for 15 drugs/drug classes compliant with the National Safety Council's Alcohol, Drugs and Impairment Division's published guidelines, including benzodiazepines, cocaine, and fentanyl. ELISA results were evaluated to determine positivity (N = 44,544), polydrug use, and popular drug combinations. Drug combination classes were adapted from the ELISA assays and grouped such that methamphetamine and amphetamine positive or opiate and oxycodone positive screens were counted as one methamphetamine and one opiate result, respectively.

Results: Overall percent positivity, percentage of cases positive for a single drug, percentage of cases positive for multiple drugs, and the top 5 drug combinations are listed in the table below. Percent positivity and the percentage of polydrug use both increased between 2019 and 2020 reaching their highest percentages in three years of 82% and 53%, respectively. Cannabis was the most identified drug across all three years with positivities between 79-80%. The popular drug combinations also remained consistent during this time with cannabinoids/benzodiazepines and cannabinoids/amphetamines being the two most prevalent combinations over all three years.

Table 1: Drug positivity, percentage of single drug and polydrug cases, and prevalent drug combinations

	2018	**2019**	**2020**
Positivity	78%	78%	82%
Single Drug	51%	54%	47%
Poly Drug	49%	46%	53%
Top 5 Drug Combinations	Cannabinoids/Benzodiazepines Cannabinoids/Amphetamines Cannabinoids/Fentanyl Cannabinoids/Cocaine Cannabinoids/Buprenorphine	Cannabinoids/Benzodiazepines Cannabinoids/Amphetamines Cannabinoids/Cocaine Cannabinoids/Fentanyl Cannabinoids/Buprenorphine	Cannabinoids/Benzodiazepines Cannabinoids/Amphetamines Cannabinoids/Fentanyl Cannabinoids/Cocaine Cannabinoids/Buprenorphine

One cannabinoid/benzodiazepine drug combination case study involved a hit and run accident. Officers observed slow and slurred speech, poor balance, lack of coordination, and droopy eyelids. A DRE evaluation revealed low blood pressure, flaccid muscle tone, and dilated pupils. The driver admitted to using several CNS depressants, including alprazolam and oxycodone, without a prescription in addition to medical marijuana. Toxicology testing confirmed the presence of cannabinoids with etizolam and alpha-hydroxyetizolam.

Conclusion/Discussion: Drug use continues to increase in drivers despite the COVID-19 pandemic. Overall percent positivity and percent of polydrug use has increased over the past three years in DUID investigations submitted to NMS Labs. Popular combinations include the most identified drug, cannabis, with benzodiazepines, amphetamines, and fentanyl.

1. Thomas, F. D. et. al. Drug and alcohol prevalence in seriously and fatally injured road users before and during the COVID-19 public health emergency. No. DOT HS 813 018. United States. National Highway Traffic Safety Administration. Office of Behavioral Safety Research, 2020.

S-030: Toxic or tolerant? A look at high drug concentrations in six impaired driving cases in Houston

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Background/Introduction: Impaired driving continues to gain attention as a major public safety concern. While interpreting blood alcohol concentrations is relatively well-defined given its per se limit, there is still much to learn about interpreting concentrations of other drugs as they are not directly related to the degree of impairment. In addition to an individual's driving behavior, appearance, and performance in standard field sobriety tests (SFST), a blood test result is also regularly used in addressing impairment. Toxicologists utilize literature, databases and other relevant information to assist in classifying a blood concentration as subtherapeutic, therapeutic/recreational, or toxic/lethal. However, due to individual differences including dose, physiological conditions, drug use history and tolerance, the boundaries between therapeutic/recreational and toxic become more blurred. Interpretation of test results in Driving While Intoxicated (DWI) cases are further complicated when extremely high drug concentrations are observed.

Objectives: To bring awareness to the forensic toxicology community about blood concentrations of certain drugs determined in DWI cases typically seen in toxic/lethal ranges.

Methods: This study examined blood toxicology results and police reports associated with impaired driving arrests in Houston, Texas from 2014 to 2020. Cases with blood concentrations higher than those reported in literature for DWI cases were selected.

Results: *Case 1:* 37Y male struck a tractor trailer and failed to stop. Officer reported slurred speech and unsteady gait. SFST results were 4/6 horizontal gaze nystagmus (HGN), 3/4 one-leg-stand (OLS), and 3/8 walk-and-turn (WAT) clues. Blood concentrations were **220 ng/mL fentanyl**, 180 ng/mL norfentanyl, 0.10 ng/mL acetyl fentanyl, and 6.3 ng/mL delta9-carboxy-THC. *Case 2:* 29Y male driving poorly in a drive-through and acting "weird". Officer reported constricted pupils, slurred speech, unsteady gait, and poor balance. Blood results were **680 ng/mL oxycodone**, 2.1 ng/mL oxymorphone, 1300 ng/mL benzoylecgonine, 7.1 ng/mL delta9-carboxy-THC, 260 ng/mL diazepam, and 59 ng/mL nordiazepam. *Case 3:* 37Y male collided with another vehicle at a stoplight. Officer reported glassy eyes, slurred speech, and poor balance. SFST results included 6/8 WAT and 4/4 OLS clues. Blood concentrations were **440 ng/mL hydrocodone**, 15 ng/mL codeine, 16 ng/mL dihydrocodeine, 5.1 ng/mL delta9-THC, 24 ng/mL delta9-carboxy-THC, 1.0 ng/mL 11-hydroxy-delta9-THC, 95 ng/mL alprazolam, 960 ng/mL MDMA, and 64 ng/mL MDA. *Case 4:* 18Y male struck another vehicle. Officer reported bloodshot eyes, slurred speech, unsteady turning and walking, and swaying. SFST results were 6/6 HGN clues. Blood concentrations were **330 ng/mL clonazepam**, 1,700 ng/mL 7-aminoclonazepam, 330 ng/mL diazepam, and 120 ng/mL nordiazepam. *Case 5:* 25Y male drove on the shoulder and failed to yield at a red light. Officer reported red, bloodshot eyes, and unsteady turning and balance. SFST results were 0/6 HGN, 2/4 OLS, and 5/8 WAT clues. Blood concentrations were **160 ng/mL delta9-THC**, 25 ng/mL delta9-carboxy-THC, and 9.7 ng/mL 11-hydroxy-delta9-THC. *Case 6:* 43Y male proceeded through a red light and collided with another vehicle. Officer reported red/glassy eyes, slurred speech, unsteady, and unstable balance. SFST results were 3/6 HGN, 2/4 OLS, and 5/8 WAT clues. Blood concentrations were **2600 ng/mL methamphetamine** and 250 ng/mL amphetamine.

Conclusion/Discussion: To the authors' knowledge these are the highest reported blood concentrations in DWI cases for fentanyl, oxycodone, hydrocodone, clonazepam, delta9-THC, and methamphetamine. The concentrations from this study are typically found in the toxic or postmortem range but considering these were from conscious drivers illustrates the difficulty in establishing toxic ranges for many drugs. High blood concentrations of drugs also suggest previous drug use and physiologic tolerance, especially with opioids. Additionally, three of the cases involved polydrug use where interpretation is further complicated by possible drug interactions. The present cases demonstrate challenges in interpretation of extremely high drug concentrations in DWI casework and that case and individual history should be considered.

PLATFORM

S-031: Same Night, Different THC DUI

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Background/Introduction: This is a case study in which one individual was stopped twice for suspected driving under the influence (DUI) within two hours. The driver, a 20-year-old male, was initially contacted on October 4th at 2330 for being parked at a closed State Park. The driver was arrested, a blood draw was completed on October 5th at 0021 and the driver was released to a ride share to go home. An hour later, on October 5th at 0121, the same DUI officer noticed the same vehicle driving at a high rate of speed and failing to use the turn signal. The driver was arrested for DUI and a second blood draw was completed on October 5th at 0201.

Objectives: The case study is an example of an individual who was stopped for DUI twice within two hours. The data shows the change in reported cannabinoid concentrations over time in the whole blood as well as the change in behavior through officer observations and performance on perform Standardized Field Sobriety Tests (SFSTs).

Methods: Whole blood samples were screened by ELISA and confirmed utilizing a liquid/liquid extraction and analyzed by LC/MS/MS for the Cannabinoids delta-9-tetrahydrocannibinol (THC), 11-hydroxy-9-tetrahydro-cannabinol (THC-OH), and 11-nor-9-carboxy-delta-9-tetrahydrocannabinol (THC-COOH). A set of six calibrators from 1.0-50 ng/mL (THC, THC-OH) and 5.0-250 ng/mL (THC-COOH) and 3 controls were concurrently analyzed with all casework. The measurement uncertainty is 19.2%.

Results: When questioned during the first stop, the driver had the keys in the ignition and the engine running. Two passengers were also present and there was a strong smell of burnt marijuana coming from the vehicle. The driver, who had watery eyes, admitted that he had just finished smoking a marijuana cigarette. While willing to perform SFSTs, the driver was unable to stay on his feet and continuously fell. Due to safety concerns, no SFSTs were performed. At the second traffic stop, the officer noted that the driver's reaction was erratic. Initially, the officer noted that the subject exited the vehicle, walked, and stood on the roadway, without difficulty; but later the officer noted that the subject's balance was unsteady. The driver performed SFSTs with the following results: Horizontal Gaze Nystagmus (HGN) 5 of 6 clues, Walk and Turn (WAT) 1 of 8 clues, One Leg Stand (OLS) 2 of 4 clues, and had watery eyes.

Draw	Time	THC (ng/mL)	THC-OH (ng/mL)	THC-COOH (ng/mL)
1	0021	62	18	169
2	0201	23	9.9	125

Conclusions/Discussion: Over time, the cannabinoid concentrations in whole blood decreased and the driver was able to attempt SFSTs during the second stop. Ultimately, the driver did go to a single trial for two charges of DUI. Based on the combination of documented findings in the police report, the toxicological results and testimony, the driver was found guilty on both counts of DUI.

S-032: A Case of Mistaken (Drug) Identity

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Background/Introduction: Recently an officer requested assistance with what he expected to be a straightforward DUID case, as the suspect displayed behavior characteristic of PCP intoxication. The officer ordered a basic blood alcohols test and ELISA drug screen, expecting PCP to screen and confirm positive. However, no drugs were detected. After consultation with a toxicologist, he expanded his request to a LC-TOF-MS screen, where dextro/ levomethorphan and its metabolite, dextrorphan/levorphanol, were detected at concentrations above those normally observed with therapeutic use. When later confronted, the suspect admitted to drinking a bottle of cough syrup. Dextromethorphan (DXM) is an antitussive, commonly found in OTC cold, flu, and cough medications such as Robitussin®, Nyquil®, and Theraflu®. For those 12 years or older, a maximum dose of 120 mg over 24 hours is recommended. However, DXM is known to be abused at higher doses, particularly by adolescents, due to the ease of accessibility and the hallucinogenic, intoxicating, and dissociative effects achieved at these higher doses. Experiences following these doses may not be euphoric; a PCP-like psychosis, including paranoia, delusions, and violent behavior, has been reported. DXM is classified as a Tier II drug and, at the time of initial circulation, was not included in the unfinalized ASB Standard 120, *Standard for the Analytical Scope and Sensitivity of Forensic Toxicological Testing of Blood in Impaired Driving Investigations*.

Objectives: This presentation will familiarize participants with the effects of DXM, particularly at higher doses, as discussed in the context of a case report. Additionally, DXM data from law enforcement cases submitted to our laboratory will be presented.

Methods: The sample was initially tested for the presence of alcohols by headspace GC and for fifteen drugs/drug classes via ELISA. Additional screening was performed by LC-TOF-MS and an additional ELISA panel, with confirmation and quantitation of DXM and metabolite performed via LC-MS/MS. Consultation with the officer and a review of arrest records were undertaken to obtain case information. Results and demographics from DXM positive law enforcement cases submitted to our laboratory between January 2010 and December 2020 were reviewed.

Results: In the presented case, the suspect was involved in a single vehicle crash when he lost control of his vehicle, striking the median guide wire of the roadway. At the scene, the suspect was combative and aggressive, ultimately assaulting an officer. Blood results from the 28-year-old male were 750±170 ng/mL dextro/levomethorphan and 91±19 ng/mL dextrorphan/levorphanol. Although the PCP ELISA was negative, the case did show decreased absorbance in comparison to other negative cases. Dextro/levomethorphan results from suspected driving under the influence of drugs (DUID) cases submitted to our laboratory between January 2010 and December 2020 primarily involved young adult males, with blood concentrations of 5.2–2300 ng/mL.

Table 1: DXM DUID Cases from 2010 – 2020

n	492
Mean DXM Concentration (± standard deviation)	261 (± 396) ng/mL
Median DXM Concentration (ng/mL)	76 ng/mL
Age (± standard deviation)	33 (± 13) years*
Gender	63% Male*

*Demographic data not available for all cases.

Drugs most frequently found in conjunction with DXM included caffeine, chlorpheniramine, THC and/or THCCOOH, and cotinine. Indication of illicit drug use, apart from THC, was uncommon.

Conclusion/Discussion: In this case, a suspected PCP intoxication was instead abuse of the cough suppressant DXM. Initial testing, which encompassed Tier I drugs, did not identify the drug responsible for the actions of the suspect as the officer was unaware of the behavior profile of DXM at high doses and no case history was provided at the time of submission. It is important to educate submitters and toxicologists that off-label use of medications may present symptoms uncharacteristic of therapeutic use.

S-033: A Determination of the Aerosolization Efficiency of Drugs of Abuse in a Eutectic Mixture with Nicotine in Electronic Cigarettes

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Background/Introduction: An e-liquid formulation is usually composed of vegetable glycerin (VG), propylene glycol (PG), an active drug such as nicotine, and flavorings. A current concern is the adulteration of e-liquids with drugs other than nicotine (DOTN). With the capabilities of an electronic cigarette (e-cig), users can virtually vaporize any thermally-stable drug, which has allowed e-cigs to become an opportune vehicle for drug experimentation. Nicotine and cannabis are the most commonly vaped drugs of abuse in e-cigs. Users are known to add other pharmacologically active substances, such as cocaine, heroin, or amphetamine. The interaction between the diluent and nicotine forms a eutectic mixture, reducing the melting point to a temperature lower than either of the individual compounds known as a eutectic point or temperature. At this point, aerosolization of the drug is promoted. During the process, aerosolization of the drugs may produce pyrolytic products, affecting the aerosolization efficiency of the drugs. This interaction was evaluated using a standardized aerosol capture method developed by the Cooperation Centre for Scientific Research Relative to Tobacco (CORESTA) E-cigarette Task Force. CORESTA is an industry standard method to allow for intra- and inter-laboratory comparisons within the e-cigarette industry.

Objectives: The purpose of this study is to determine the effect of nicotine in the aerosolization and recovery of methadone via an automated vaping machine using parameters adopted from the CORESTA method. A GC-MS method was validated to determine the experimental concentrations of the aerosolized drugs and determine the drug recovery. The objective is to characterize the effects of drug interactions, specifically with nicotine, in the e-cigarette environment.

Methods: Calibration curves for nicotine and methadone were validated accorded to the AAFS ASB Standard for Forensic Toxicology Method Validation over five days. The linear ranges were evaluated using quality controls and assessed for bias, precision, and carryover. Aerosol capture was performed on four e-liquids using CORESTA-defined parameters on an automated vaping machine: pure nicotine and methadone hydrochloride at 12 mg/mL in 50:50 PG:VG, 1:1 methadone hydrochloride:nicotine at 12 mg/mL in 50:50 PG:VG, and 50:50 PG:VG as a blank. These samples were analyzed with a Gas Chromatograph-Mass Spectrometer to determine the amount aerosolized and percent recovery of the drug. Additionally, the aerosolized samples were screened for the pyrolytic products of nicotine and methadone: β-nicotyrine and EDDP, respectively.

Results: The parameters for the aerosol capture method were set at an inhale duration of 3 seconds, an exhale duration of 10 seconds, a puff volume of 60 mL, and the number of puffs at 15 puffs. In the single drug e-liquids prepared at 12 mg/mL, 0.22 g ($\pm$8 %CV) of nicotine was aerosolized while 0.31 g ($\pm$4 %CV) of methadone hydrochloride was aerosolized (N = 3). The amount of combined drug aerosolized in the nicotine and methadone mixture increased to 0.37 g ($\pm$3 %CV). The nicotine e-liquid had a recovery of 73% ($\pm$21 %CV) and the methadone hydrochloride e-liquid had a recovery of 99% ($\pm$2 %CV; N = 3). The 1:1 methadone hydrochloride:nicotine mixture showed recoveries of 110% ($\pm$6 %CV) and 87% ($\pm$5 %CV) for methadone hydrochloride and nicotine, respectively. Also, the aerosolized samples showed the presence of their respective pyrolytic products in all the e-liquids.

Conclusion/Discussion: E-liquids containing nicotine and DOTN, creating a eutectic mixture, can increase the amount of drug delivered in the aerosol. The increased drug dosages could result in untoward effects. The presence of the pyrolytic products can also present adverse effects.

PLATFORM

S-034: Impact of Vaping Ethanol on the Roadside Field Sobriety Test in a Clinical Setting

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Background/Introduction: Recent studies have shown that electronic cigarette (e-cig) liquid formulations (e-liquids) can contain ethanol, sometimes in concentrations greater than 20%. Ethanol, rarely listed as an ingredient in e-liquids, is often used as a thinning agent, to dissolve insoluble substances, or added intentionally for consumption. The ethanol has been demonstrated to be aerosolized by e-cigs. The Standardized Field Sobriety Test (SFST) evaluates several operant conditions to assess ethanol impairment, following an observation of cues while a vehicle is in motion. The Preliminary Breath Tests (PBT) and Evidentiary Breath Tests (EBT) are conducted to measure ethanol in exhaled breath, with a 15-20 minute wait period. An evaluation of vaping ethanol from e-liquids is essential for law enforcement, medical review officers, ethanol treatment and recovery providers, workplace drug testing, vehicle ignition interlock devices, and court mandated drug testing to understand potential ramifications.

Objectives: Under VCU IRB HM20015064 and in collaboration with the Virginia Department of Forensic Science, the Center for the Study of Tobacco Products at VCU, VCU Police Department, and City of Richmond Police Department, this study was designed to evaluate ten users' perception of impairment from vaping ethanol using a participant subjective assessment survey and assess the indicators of impairment using the SFST, PBT, and EBT.

Methods: Participants were enrolled into the study based on inclusion and exclusion criteria. They vaped either one or ten puffs of an e-liquid (0% or 20% ethanol). Participants answered a computerized survey for self-assessment of impairment. Law enforcement officers (LEO) assessed indicators of impairment by following a study design incorporating SFSTs, PBTs, and EBTs at predetermined intervals.

Results: PBT administration resulted in positive ethanol findings as high as 0.037 g/210 dL immediately after ten-puff and one-puff scenarios using an e-liquid with 20% ethanol. The ten-puff scenario produces slightly higher results than the single puff. No ethanol or instrument errors were indicated by subsequent PBTs or EBTs. Impairment was not indicated by SFST after use of any ethanol concentration. Vaping 20% ethanol e-liquids slightly increased self-perceived impairment.

Conclusion/Discussion: A well-designed human clinical study with robust relevant collaborations between a crime lab, police departments, and a research university is important to address pertinent questions to forensic science. Preliminary data suggested the standard wait period of 15-20 minutes employed by LEO before administering a PBT for roadside stops was effective in negating vaping-related false positive ethanol results. Additionally, preliminary data suggests vaping ethanol does not significantly impact SFST results but may affect individual's perceived experience.

PLATFORM

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S-035: Mitragynine: A Postmortem Case Study Perspective (2018-Present)

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Background/Introduction: *Mitragyna Speciosa*, colloquially known as "Kratom", is a plant native to Southeastern Asia used for its medicinal properties as a dietary and herbal supplement. Mitragynine, the predominant psychoactive alkaloid present in Kratom products, produces stimulant-like effects at low doses and opioid-like effects at higher doses leading to an increase in recreational use as a "legal high". Although there are purported benefits of the use of Kratom products for the management of pain and opioid withdrawal symptoms, the safety of the products at this time is relatively unknown. In 2016, the U.S. Food and Drug Administration issued warnings to consumers to not use Kratom, with intentions of scheduling the drug. However, mitragynine remains unscheduled and companies still market Kratom as a miracle herb that is as harmless as caffeine. These products remain readily available, legally, for consumers to purchase.

Objectives: This presentation will evaluate the prevalence of mitragynine in postmortem forensic toxicology casework performed at NMS Labs from January 2018 through February 2021. Blood concentrations and positivity rates will be reviewed and specific cases will be presented where mitragynine was a significant finding.

Methods: Routine postmortem testing with mitragynine confirmation began at NMS Labs in 2018. Samples were screened via Liquid Chromatography/Time of Flight Mass Spectrometry (LC-TOF/MS). Confirmatory analysis of cases screening positive for mitragynine was conducted by Liquid Chromatography/Tandem Mass Spectrometry (LC-MS/MS) with an analytical measurement range of 5.0 – 1000 ng/mL in blood. Results were compiled for postmortem blood to analyze positivity rates and potential trends in mitragynine use.

Results:

	2018	2019	2020	2021*
n	747	1054	1610	196
Mean (+/- SD) (ng/mL)	362 (± 638)	335 (± 677)	419 (± 818)	445 (± 933)
Median (ng/mL)	130	110	140	100
Range (ng/mL)	5.9 - 7000	5.4 - 8900	5 - 11000	5.5 - 8000
Positivity Rate (%)	1.10	1.18	1.87	1.90

*Data collected through February 2021

The increase in popularity of Kratom use in Western society is reflected in the positivity rates found in casework. There was an approximate 77% increase in the positivity rate of mitragynine from 2018 to 2020. A wide variety of concentrations can be observed; approximately 20% of cases had concentrations greater than or equal to 500 ng/mL. Toxic blood concentration ranges have not been well established for mitragynine due to a number of confounding factors such as polypharmacy and underlying pathology. Further, mitragynine exhibits limited stability, so pre-analytical factors may also play a role when interpreting blood concentration. Despite the persistent view that mitragynine is "harmless", it is still being detected in toxicological casework; therefore, the role of mitragynine in postmortem casework requires thoughtful assessment. Case studies will be presented in further detail during the presentation, with a focus on cases where mitragynine was found at elevated concentrations with limited co-positivity.

Conclusion/Discussion: The prevalence of mitragynine in postmortem toxicology casework represents a potential public health threat. The aim of this study is to highlight the increase in prevalence of mitragynine over time, in addition to the careful assessment of postmortem cases involving elevated blood mitragynine levels.

PLATFORM

S-036: Analysis of Umbilical Cord Tissue as an Indicator of In Utero Exposure to Toxic Adulterating Substances

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Background/Introduction: *In utero* drug exposure is a significant public health threat to the well-being and normal development of the neonate. More recently, testing of umbilical cord tissue (UCT) has been employed to measure illicit drug exposure. It has been demonstrated that drugs used by the mother during the third trimester may be retained in the cord tissue. Detection of drugs in the tissue can then be used to identify neonates for follow-up monitoring to assess potential health impacts. Recent attention has also been given to potential adverse health effects among drug users, resulting from exposure to pharmacologically active adulterants and cutting agents in the street drug supply. The *in utero* effects of these substances have not been well studied in humans, nor has their presence previously been demonstrated as a means for assessing adverse health effects in the neonate. Here, we describe the application of a novel test method to analyze UCT for the presence of common adulterating substances, including acetaminophen, aminopyrine, caffeine, dextromethorphan, dipyrone (metabolites), ketamine, lidocaine, levamisole, phenacetin, promethazine, quetiapine, quinine, and xylazine.

Methods: Commonly identified adulterating and/or cutting agents from recent studies/analyses of seized drug exhibits were used as the basis for the scope of this analysis. De-identified human UCT samples, which had previously tested positive for either cocaine (benzoylecgonine) or opiates (6-MAM, morphine, &/or meconin), were utilized. Tissue samples were rinsed, cut, homogenized, and underwent an SPE extraction procedure. Samples were run blank-and-spike with a mixture of the target analytes. Analysis was performed using LC/Q-TOF with results being reported qualitatively.

Results: In total, 300 UCT samples, collected in 2017 and 2019 from locations across the United States, were analyzed using the method described. From this sampling, 183 (61%) were identified as positive for cocaine and 117 (39%) for opiates. No samples were positive for both cocaine and opiates. The UCT samples were analyzed for more than 20 common adulterating and/or cutting agents. The results of the top five findings are shown in the table below along with positivity rates. Caffeine and lidocaine were the most frequently identified compounds individually, and in combination with each other. In general, positivity rates of individual compounds were similar between the cocaine and opiate test groups, apart from diphenhydramine, promethazine, and levamisole.

Compound	% Adulterant in Total Cohort	% Adulterant in Cocaine Positives	% Adulterant in Opiate Positives
Caffeine	76	74	79
Lidocaine	72	75	67
Diphenhydramine	27	30	22
Quetiapine	24	24	25
Levamisole	12	18	3

Conclusion/Discussion: Many of the adulterants used in the street drug supply also have legitimate medicinal and/or therapeutic uses, including several of the compounds most frequently detected in this study. When used in moderate quantities, these legitimate-use compounds are unlikely to elicit toxic effects. However, repeated exposure to variable and often high levels of these compounds, as is present in illicit drug materials, increases the risk of adverse effects on both the mother and the fetus/neonate. Less frequently detected adulterants with no legitimate therapeutic use such as phenacetin, xylazine and dipyrone (metabolites) were present in 5%, 1%, and 0.3% of cases respectively.

Importantly, our data demonstrates that substances other than traditional drugs of abuse can be detected in UCT. These drugs can act as markers for *in utero* exposure to toxic adulterating substances during gestation. While there is cause for concern with respect to any unintentional drug use, illicit drug use during pregnancy, including uncontrolled dosing, poly-adulterant consumption, and the interactions of these drug mixtures, produces a significant public health threat to the neonate which is still far from being understood.

S-037: Postmortem investigations in a death involving anabolic steroid: hair is the key specimen

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Background/Introduction: Anabolic steroids are synthetic derivatives of the endogenous testosterone. These drugs are abused because it has been claimed that they increase lean body mass, increase strength, increase aggressiveness, increase endurance and lead to a shorter recovery time between workouts. Numerous side-effects after long-term anabolic steroids abuse have been described, and include increase in blood pressure, alterations of the cardiovascular system, liver toxicity, acne, atrophic testes and sexual dysfunction. When abuse is prolonged, subjects can develop psychiatric effects, which include antisocial lifestyle, psychosis, low self-confidence, childhood conduct disorders, delirium, depression and aggressiveness. The prolonged abuse of anabolic steroids is associated with cardiovascular diseases, including cardiac hypertrophy, myocardial infarction and sudden cardiac death.

Objectives: Because anabolic steroids are seldom tested in forensic laboratories, and even less in hair specimens, the present case aims to present the analytical strategy of the laboratory when a cardiac death is submitted.

Methods: A 59-year old male was found dead at home, with 2 empty vials of an oily preparation attributed to a manufacturer from East Europe. There were no labels on the vials, probably due to an on-line purchase. The subject was a former weightlifter, also known as an anabolic steroids abuser. The local prosecutor ordered a body examination, which was unremarkable and allowed collecting femoral blood, urine and scalp hair (6 cm, brown). He was treated for cardiac insufficiency with quinidine. Biological specimens were submitted to standard toxicological analyses including a screening with liquid chromatography couplet to accurate mass spectrometry, but also to a specific LC-MS/MS method for anabolic steroids testing.

Results: Autopsy findings were un-conclusive. Ethanol tested negative in blood and urine. HbCO was 1.2 % and cyanides were at physiological concentrations (<80 ng/mL). ELISA screenings were negative for pharmaceuticals and drugs of abuse. Specimens were free of drugs of abuse, including NPS, using LC-MS/MS screening methods. In blood, only endogenous steroids were found, i.e., testosterone (<1 ng/mL), dihydrotestosterone (<1 ng/mL) and dehydroepiandrosterone (<1 ng/mL). These concentrations were physiological. No synthetic anabolic steroid was identified. Quinidine was therapeutic, at 791 ng/ml. On the opposite, the hair test results were highly indicative of long-term steroids abuse, with the identification of several drugs, including testosterone (140 pg/mg), testosterone propionate (605 pg/mg) and testosterone decanoate (249 pg/mg), but also boldenone (160 pg/mg), trenbolone (143 pg/mg) and metandienone (60 pg/mg).

Conclusion/Discussion: As the autopsy was not informative enough, the positive findings of steroids in hair was of paramount importance to establish the cause of death**.** The presence of several different steroids can be explained by a special regimen, a pattern named cycling, i.e. use of steroids over a specific period of time followed by a abstinence period, again followed by a new period of use. In addition, as each steroid has its specific presentation (oral or injectable) and its specific pharmacology with more or less androgen effects and increase in muscle building, a mix between several agents is recommended to enhance the benefit of the administration. Toxicologists and forensic pathologists should be aware of possible abuse of steroids proposed as image- and performance-enhancing drugs to promote body changes and nice appearance. Steroid agents can also be used to lose weight or to enhance athletic performance, even in subjects older than 50. The possibility of cardiac insufficiency in a steroid abusing subject is a forensic situation that has to be documented by a comprehensive toxicological screening, including anabolic steroids.

S-038: Postmortem Oral Fluid Drug Testing in Alabama

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Background/Introduction: In 2018, the Alabama Department of Forensic Sciences (ADFS) established the first comprehensive DUI/D oral fluid (OF) drug testing program in the United States to include approved roadside screening devices and evidentiary, confirmation testing at the laboratory. Due to its non-invasiveness and ease of collection, OF drug testing now spans a wide number of application including workplace, pain management, and DUI/D testing. We have previously shown that drugs with short half-lives such as cocaine, THC, and heroin are more readily detected in oral fluid than blood in DUI/D cases. The presence of active, parent drug, low matrix interference, OF: blood ratios > 1 for many drugs, and oral cavity coating/contribution makes OF an attractive specimen for death investigations. However, the use of OF drug testing in postmortem toxicology has not been extensively researched.

Objectives: To investigate the prevalence of drugs of abuse in oral fluid specimens collected during autopsy from suspected overdose cases.

Methods: ADFS medical examiners in Montgomery, Mobile, and Huntsville collected approximately 50 OF and blood samples from suspected overdoses at autopsy. Quantisal OF collection devices, previously validated for OF DUI/D applications, were used to swab the oral cavity of deceased subjects. OF and blood specimens were screened using two technologies: immunoassay by Randox Evidence Analyzer and high-resolution mass spectrometry with an Agilent 6545 Q-TOF. OF confirmation testing was conducted using two previously validated methods on an Agilent 6470 LC/MS/MS. The first method identified 20 drugs of abuse following an in-tip Dispersive Pipette extraction (DPX) technique using a Hamilton Starlet automated extraction system. Furthermore, a liquid-liquid extraction detected cannabinoids (e.g. delta-9-THC, delta-8-THC, THC-OH, THC-COOH, CBN, CBD, and CBG). Blood confirmations were performed by liquid-liquid or solid phase extraction and GC/MS or LC/MS/MS. Positivity rate, OF:blood ratios, and median concentrations for the most commonly detected drugs (i.e. fentanyl, methamphetamine, 6-MAM) in OF and blood were calculated.

Results: Comparable positivity rates for oral fluid and blood were noted for most drugs of abuse. 6-MAM and fentanyl had a higher prevalence in oral fluid than blood (Table 1). Semi-quantitative results were collected for research purposes. OF: blood ratios from postmortem specimens were compared to previously calculated OF: blood ratios for DUI/D cases. Postmortem OF:blood ratios displayed larger variation and were close to unity or <1.0 for targets in many cases. The Q-TOF screen enhanced scope of analysis and facilitated the detection of novel psychoactive substances such as fentanyl analogues and designer benzodiazepines in both specimen types.

Table 1 – 6-MAM and Fentanyl Results

	6-MAM (OF)	6-MAM (Blood)	Fentanyl (OF)	Fentanyl (Blood)
Positivity Rate (%)	100% (7/7)	38% (3/8)	95% (20/21)	85% (22/26)
Mean (ng/mL)	52	46	51	13
Median (ng/mL)	62	21	43	12

Conclusion/Discussion: Drugs of abuse were readily detected in OF specimens collected at autopsies utilizing methodology previously developed and validated by ADFS for OF DUI/D testing. Oral fluid proved to be a useful specimen in distinguishing between heroin abuse and morphine pain medication use when 6-MAM was not detected in the blood and other specimens. In conclusion, oral fluid may serve as an excellent supplemental specimen to blood providing more information on drug use in suspected overdoses or poisoning cases.

S-039: Identification and quantification of ketamine derivatives 2-fluorodeschloroketamine, deschloro-N-ethyl-ketamine, deschloroketamine and ephenidine, along with clonazolam, etizolam and other NPS in seized materials and hair: A French case-study

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Background/Introduction: The emergence of New Psychoactive Substances (NPS) in the drug market is a worldwide problem. A 24-year-old man has been followed for several years for drug abuse. Seven powders and two stamps he bought over the Internet, as well as a sample of his dark brown hair were sent to our laboratory for toxicological investigations.

Objectives: To provide an insight into the nature and purity of seized materials containing NPS that circulated in France in 2020, and to determine hair concentrations found after regular use.

Methods: Liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS) or high resolution mass spectrometry (LC-HRMS) were used in the present casework (1, 2). One mg of each powder was dissolved in 1 mL of methanol, and each 9mm^2 stamp was macerated in 2 mL of methanol. Ten microliters of diluted solutions in the mobile phase were injected onto the system. The hair was washed twice with dichloromethane, segmented (A 0–3 cm, B 3–6cm, C 6–9cm) then extracted with a mixture of solvents. Targeted screening by LC-MS/MS was performed in MRM mode on a TSQ Endura (triple quadrupole) while untargeted screening by LC-HRMS was achieved in data-dependent mode (ddms2) on a Q-Exactive (Orbitrap), both devices were from Thermo Fisher Scientific®.

Results: Six analytes were identified in powders: ephenidine (100%, n= 2), 2-fluorodeschloroketamine (2F-DCK: <0.1—96%, n= 5), deschloroketamine (DCK: <0.1-81.5% n= 5), 5-methoxy-dimethyltryptamine (5-MeO-DMT: <0.01%, n= 1), 3/4-MeO-PCP (0.35%, n= 1), phenibut (not quantified, n= 1), and two analytes from the two stamps: clonazolam (2.1%, n= 1) and etizolam (3.6%, n= 1). The products contained from 1 to 4 analytes. The labeling corresponded to the major substance identified in each product. The low levels of NPS observed in some products (≤ 2.5%) were mainly related to cross-contamination by containers exchanged by the patient. Hair analysis allowed the quantification (pg/mg) of: 2F-DCK (3260/4650/6702), DCK (885/1500/1850), ephenidine (230/128/190), 5-MeO-DMT (1/15/17), clonazolam (10/24/36), etizolam (5/5/11), as well as deschloro-N-ethyl-ketamine (13/27/36), which was not present among the analyzed products. 3/4-MeO-PCP was not detected in hair, possibly due to its low amount in the analyzed powder (0.35%). The few existing literature data report hair concentrations ranging from 7 to 340 pg/mg of 2F-DCK (n = 3) and from 37 to 107 pg/mg of etizolam (n = 5), which is consistent with chronic use of 2F-DCK and occasional use of etizolam in our patient. To our knowledge, this is the first case describing hair concentrations of DCK, deschloro-N-ethyl-ketamine, ephenidine, 5-MeO-DMT and clonazolam, reflecting regular exposure to these substances in the 9 months preceding the sampling. Finally, it should be noted that phenibut that was formally identified but not currently quantified in hair is a GABA agonist scheduled as a psychotropic drug in France since 2020.

Conclusion/Discussion: This case illustrates the circulation of new designer drugs in France in more pure formats, that were cut inadvertently by the consumer himself, which constitutes an additional risk of unexpected toxic effects. Hair analysis still shows its relevance as a tool for long-term monitoring of NPS users.

[1] : I.A. Larabi *et al.* Prevalence of New Psychoactive Substances (NPS) and conventional drugs of abuse (DOA) in high-risk populations from Paris (France) and its suburbs: A cross sectional study by hair testing (2012-2017). *Drug Alcohol Depend*, 2019, *204*, 107508. [2] : N. Fabresse *et al.* Development of a sensitive untargeted liquid chromatography-high resolution mass spectrometry screening devoted to hair analysis through a shared MS2 spectra database: A step toward early detection of new psychoactive substances. *Drug Test Anal*, 2019, *11*, 697–708.

PLATFORM

S-040: Placenta as an alternative matrix to detect cannabis exposure during pregnancy

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Background/Introduction: Cannabis consumption has been increasing worldwide among pregnant women. According to the 2017 National Survey on Drug Use and Health, 7% of pregnant women used cannabis in the past month, a 1.3% increase from the 2014 survey. As a result of the increase in legalization and decriminalization of marijuana at the state level across the US, this upward trend is expected to continue rising. Due to the negative effects of prenatal cannabis exposure and its clinical and forensic implications, it is necessary to develop objective, sensitive and specific methods to determine cannabinoids use during pregnancy. Although there have been methods published for the detection of cannabis exposure by other neonatal matrices (i.e. meconium, umbilical cord), the investigations using placenta are scarce.

Objectives: The objective of this work was to develop a novel method for the determination of 3 cannabinoids, Δ9-tetrahydrocannabinol (THC), cannabinol (CBN) and cannabidiol (CBD), and 5 THC metabolites, 11-hydroxy-THC (THC-OH), 11-nor-9-carboxy-THC (THCCOOH), 8-β-11-dihydroxy-THC (THC-diOH), THC-glucuronide and THCCOOH-glucuronide, in placenta through liquid chromatography-tandem mass spectrometry (LC-MS/MS). This method was then applied for the analysis of authentic placenta samples from newborns exposed to cannabis during pregnancy.

Methods: Placenta samples (0.5 ± 0.02 g) were cut into small pieces and homogenized in methanol in a bead mill. The samples were centrifuged, and the supernatant was extracted by mixed-mode cation exchange solid-phase cartridges. Chromatographic separation was performed on a Kinetex F5 column, 100x2.1 mm, 1.7 μm (Phenomenex) at 40°C, with a gradient of 0.1% formic acid in water and 0.1% formic acid in acetonitrile at a flow rate of 0.5 mL/min. The total run time was 14 min. Two transitions per analyte were monitored in multiple reaction monitoring (MRM), using electrospray ion source in positive mode. Nineteen authentic placenta samples from cannabis-exposed newborns, which had matching meconium samples previously tested positive for cannabinoids, were analyzed.

Results: The method was validated following ANSI/ASB Standard 036. The method linearity was 0.5-100 ng/g for THC- and THC-COOH-glucuronides, 5-100 ng/g for THC-OH, THCCOOH, THC, CBD and CBN, and 20-100 ng/g for THC-diOH. Inaccuracy was from -8.1% to 17.5% (n=12), and imprecision <19.5% (n=12). The extraction efficiency ranged from 16% (THC-diOH) to 71.1% (THCCOOH). Significant ion suppression was detected for all the compounds (from -90.8% to - 58%), except for THCCOOH-glucuronide (-24.6%), showing %CV 5.4%-37.1% (n=10). No endogenous interferences were detected (n=10). Seventeen out of the 19 placenta samples tested positive for cannabinoids. All of them were positive for THCCOOH-glucuronide (median 8.3 ng/g, range 0.5-28 ng/g), and two cases also tested positive for THCCOOH (4.3 and 7.5 ng/g). The predominant metabolites in meconium samples (n=19) were free THCCOOH (15.2, 3.9-145.6 ng/g) and THC-diOH (47.6, 5.4-887.4 ng/g). The cannabinoids CBD (n=14) and CBN (n=13) were detected in meconium samples, but not in any of the paired placenta samples. In 2 cases, meconium was positive, and placenta was negative. In these 2 cases meconium was positive only for THC-diOH, and at low concentrations, 8.2 and 9.3 ng/g.

Conclusion/Discussion: The method developed was specific, sensitive and accurate for cannabinoids, including THC- and THC-COOH-glucuronides, in placental tissue by LC-MS/MS. Agreement between data obtained from authentic placenta samples and from meconium of correlating cases demonstrates the effectiveness of using placental tissue to detect cannabis in utero exposure. Based on the analysis of authentic samples, THCCOOH-glucuronide is the recommended biomarker in placenta to uncover cannabinoid exposure during pregnancy.

PLATFORM

S-041: Interlaboratory Comparison of Blinded Hair Testing Reference Samples

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Background/Introduction: Hair**,** being a solid and inhomogeneous matrix open to the elements, offers a different set of analytical challenges compared to urine, blood and oral fluid. Among these is the extent to which external contamination could contribute to drug levels in hair. Given the many modes of drug incorporation and contamination in hair, decontamination is a challenging task and it is likely that decontamination protocols remove both drugs from external contamination and from drug use. Decontamination and extraction procedures in hair testing laboratories vary widely making interlaboratory comparisons through traditional proficiency testing difficult.

Objectives: The main objective of this study was to compare results from five commercial hair testing laboratories using hair reference material samples. Laboratories were evaluated for the ability to decontaminate the sample and reliably measure drug incorporated from use. The performance of the laboratories was evaluated in light of the proposed mandatory guidelines published in the federal register on 9/10/2020 for bias, precision, selectivity, and decontamination efficiency.

Methods: A series of reference samples were prepared at RTI International and submitted as blind samples to five commercial hair testing laboratories who tested the samples using their routine procedures. A total of nine samples including certified negative, authentic drug user samples positive for cocaine, and drug-contaminated hair (washed and unwashed) were prepared and submitted in five replicates to each laboratory. Reference material samples using hair from drug users were prepared and aliquoted according to reference material production protocols accredited under ISO 17034. Negative hair was contaminated with cocaine or methamphetamine either by soaking for an hour in a 3 µg/mL solution or by rubbing drug powder into the hair (at 1.25 mg/g hair) followed by application of artificial sweat and drying.

Results: All laboratories correctly identified cocaine in samples from drug users and correctly identified the certified negative hair as negative. Hair contaminated with cocaine solution (unwashed) presented challenges to two laboratories and hair contaminated with cocaine powder (washed) resulted in positives at all laboratories. Cocaine user hair contaminated with methamphetamine both washed and unwashed resulted in positive results at most laboratories. The laboratories had differing approaches to identification of cocaine positive samples, with some requiring presence of benzoylecgonine and/or hydoxycocaine. Qualitative results are presented in the table (unintended positive results bolded). Within laboratory quantitative results were acceptable with between day CV's at or below 11% for most laboratories. However, the results appear to be method dependent and reported cocaine concentrations varied five-fold between laboratories.

Sample	Intended Result	Contaminant	Lab A	Lab B	Lab C	Lab D	Lab E
1	Negative	None	Negative	Negative	Negative	Negative	Negative
2	Cocaine(+)	None	Cocaine(+)	Cocaine(+)	Cocaine(+)	Cocaine(+)	Cocaine(+)
3	Cocaine(+)	None	Cocaine(+)	Cocaine(+)	Cocaine(+)	Cocaine(+)	Cocaine(+)
4	Negative	Cocaine Solution	**Cocaine(+)**	Negative	Negative	**Cocaine(+)**	Negative
5	Negative	Cocaine Solution/washed	Negative	Negative	Negative	Negative	Negative
6	Negative	Cocaine Powder	**Cocaine(+)**	**Cocaine(+)**	**Cocaine(+)**	**Cocaine(+)**	Cocaine Unsuitable
7	Negative	Cocaine Powder/washed	**Cocaine(+)**	**Cocaine(+)**	**Cocaine(+)**	**Cocaine(+)**	**Cocaine(+)**
8	Cocaine(+)	MAMP Solution	Cocaine(+)/ **MAMP(+)**	Cocaine(+)/ **MAMP(+)**	Cocaine(+)/ **MAMP(+)**	Cocaine(+)/ **MAMP(+)**	Cocaine(+); MAMP Unsuitable
9	Cocaine(+)	MAMP Solution/washed	Cocaine(+)/ **MAMP(+)**	Cocaine(+)	Cocaine(+)	Cocaine(+)/ **MAMP(+)**	Cocaine(+); MAMP Unsuitable

Conclusion/Discussion: This study has shown that it is possible to produce and distribute positive and/or contaminated hair samples to laboratories for proficiency testing. In such a setting, dry contaminated samples might be the best model for contamination, as

results appear similar to those from in situ contamination. It is also evident that laboratories generally provide hair testing services of high quality, but that some challenges remain. While the laboratories used precision methodology that provided consistent and reproducible results within each laboratory, large differences between laboratories were demonstrated and decontamination was inconsistent.

S-042: Evaluation of Extraction Parameters for the Analysis of Three Authentic Hair Reference Materials (HRM) Using Statistical Design of Experiments (DoE)

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Background/Introduction: There are many differing opinions regarding the optimal methods for hair analysis, particularly regarding pretreatment parameters. Extraction methods include enzymatic, acid/base, and solvent techniques, each with their own advantages and disadvantages. Other pretreatment parameters include incubation times, temperatures, and size of the extracted hair particles. To assess pretreatment parameters for drugs of abuse, the statistical technique known as DoE is useful. DoE allows for evaluating both the individual roles and the combinatorial associations between multiple variables and drug extraction efficiency. Previous research in this laboratory has focused on incorporated hair reference material (HRM), which is prepared in the laboratory by incubating drug-free hair in a solution with the drug of interest present at specified concentrations, but this approach does not necessarily reflect the mechanism by which drug incorporates into hair *in vivo*. In addition, with incorporated HRM, some drugs may not be incorporated into hair at high enough concentrations for use as a standard. In contrast, authentic HRM, which is prepared by mixing hair from drug users to achieve specific drug concentrations, is an effective standard for drug testing, since drug is incorporated into the hair through natural processes of the body.

Objectives: The objective of this work was to use DoE to determine trends in the best practice methods for extraction of methamphetamine, oxycodone, diazepam and nordiazepam, alprazolam, cocaine and metabolites, morphine and metabolites, and fentanyl from authentic HRM.

Methods: Authentic HRM containing the drugs of interest, with concentrations ranging from 10-5,000 pg/mg, was obtained from RTI, Inc. Samples of 20 mg each were weighed into 1.8 mL steel milling jars. The samples underwent extraction parameters determined using a 2^3 full factorial DoE matrix. Each sample was randomly assigned specific design points made up of combinations of factors of interest in extraction protocols. These included extraction solvent/sample size ratio (12.5 or 25 µL/mg), particle size (pulverized into a powder using a Retsch® MM200 ball mill with chrome-steel milling beads at 3,800 rpm for 30 s or cut into snippets with scissors), and extraction time (2 or 24 h). The samples were extracted using a solvent swelling technique in which the hair was incubated in a methanol:acetonitrile:2 mM ammonium formate solution (25:25:50) at 37°C. The samples were then transferred into 2 mL Eppendorf tubes and centrifuged for 30 min. Post-centrifugation, the eluent was subjected to solid phase extraction using an Agilent® Bond Elut LRC mixed mode C_8 and strong cation-exchange (SCX) cartridge. An Agilent® 1290/6460 LC-QqQ-MS was used for analysis with an Agilent 1.8® µm Zorbax Eclipse Plus C_{18} rapid resolution HD column (2.1 x 50 mm; 1.8 µm).

Results: Analysis of variance (ANOVA) f-tests were performed post-analysis to determine if different extraction parameters resulted in significantly different results. A consensus statement was made based on the design points with the highest percent recovery to determine which parameters were most effective for extraction of the drugs of interest. Data indicated that the most effective method for extracting each of the drugs of interest from authentic HRM consisted of pulverizing the hair into a powder prior to a 2 h extraction using a 12.5 µL/mg extraction solvent to sample size ratio.

Conclusion/Discussion: The use of DoE allowed for the determination of an optimized method for extracting drugs of interest from authentic HRM. Additionally, based on the ANOVA f-tests, it was determined that studying forensic hair analysis variables individually and in combination with each other is critical, and DoE is a valuable approach for this purpose. Finally, the application of DoE to forensic hair analysis has the potential for identifying consistent standard procedures in hair testing, allowing the forensic science community to better understand the value, reliability, and validity of hair testing as a technique for drug detection.

PLATFORM

S-043: Effects of Fentanyl on Blow Fly Development and Quantitation of Fentanyl from Insect Tissue via QuEChERS Extraction and LC-MS/MS Analysis

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Background/Introduction: After 72 hours since death, entomological estimation of a minimum time since death is one of the most accurate techniques due to the predictable growth of insects. In the presence of drugs however, this development can be altered. While many drugs have been explored in entomotoxicology studies, little is known about the effects of fentanyl on the growth and development of blow flies. With fentanyl having an impact on the opioid epidemic, it is important to understand the effects the drug can have on the development of insects.

Objectives: Evaluation of developmental effects of fentanyl on larva and pupa (mass, length, and width) of blow flies *Lucilia sericata* (Meigen) (Diptera: Calliphoridae). Evaluation of persistence and quantitation of fentanyl and metabolites from larva and pupa tissue after feeding on liver tissue with the presence of fentanyl. This work aims to validate a quick, easy, cheap, effective, rugged, and safe (QuEChERS) extraction procedure for the quantitation of fentanyl and metabolites from larva and pupa tissue using tandem mass spectrometry.

Methods: Fentanyl was spiked into 20 g aliquots of human liver tissue to evaluate four levels: control (0 ug/kg), low (10 ug/kg), medium (100 ug/kg), and high (350 ug/kg) concentrations. Each level was created in duplicate, one for larva (day 4 collection) and the other for pupa (day 12 collection). To each aliquot of liver, approximately 50-60 eggs (by mass) were placed and allowed to feed until time of collection. Insects were collected from the first replicate at day 4 (larva) and insects were collected from the second replicate at day 12 (pupa). To evaluate developmental effects, the mass, length, and width of the collected insects were documented. Metrics also evaluated were survivor rates, percent stage, and percent pupated. Statistical analysis for the physical characteristics were performed using JMP Pro 15.1.0. Insects from each level and day of collection were randomly selected and extracted using the validated QuEChERS extraction. Liver to insect concentration ratios were calculated for fentanyl when detected. For the larva, a correlation of liver concentration to insect concentration was calculated. A modified QuEChERS extraction was validated and used to quantify fentanyl and metabolites (norfentanyl, 4-ANPP, and β-hydroxy fentanyl) from larva and pupa tissue. An Agilent 6470 LC-MS/MS system was used for quantitation and a Zorbax Eclipse Plus C18 RRHD 3.0x100 mm, 1.8 um column utilized.

Results: Survivor rates for the control, low, and medium treatments ranged from 63.6% to 95.4% for the larva and pupa collections. The survivor rates for high treatment (350 ug/kg) were drastically lower at 28.8% and 12.0% for the larva and pupa collections respectively. The evaluation of physical characteristics for the larva (day 4 collection) showed a statistical difference ($p<0.05$) between the treatments low (10 ug/kg) and medium (100 ug/kg) in comparison to the control for mass, length, and width. Average fentanyl concentrations for the larva tissue were 1.3 ± 0.4 ug/kg, 11.6 ± 5.0 ug/kg, and 26.9 ± 12.8 ug/kg for the low, medium, and high treatments respectively. Average fentanyl liver to larvae concentrations were 7.5, 8.1, and 9.1 for the low, medium, and high treatments respectively. Fentanyl was detected in the pupa tissue in the medium and high treatments only in concentrations averaging 0.14 ± 0.04 ug/kg and 0.32 ± 0.12 ug/kg, respectively.

Conclusion/Discussion: The comprehensive extraction and LC-MS/MS method was proven applicable to authentic insect specimens that fed on liver tissue in the presence of fentanyl. Fentanyl had a significant impact on the development of the insects as displayed in reduced survivor rates in the highest treatment and in significant differences in the physical characteristics of the insects.

Keywords: QuEChERS, Fentanyl, Entomotoxicology, *Lucilia sericata,* Mass Spectrometry

S-044: Determination of Cannabinoids in Meconium

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Introduction: Marijuana's main known psychoactive constituent, Δ9-tetrahydrocannabinol (Δ9-THC), is a federally Schedule 1 drug. Several states have legalized marijuana for medicinal and recreational use. Cannabidiol (CBD), another constituent of marijuana, was legalized with the passage of the 2018 Farm Bill. A vast array of products containing Δ9-THC and/or CBD are available. In a 2015 survey, 4.7% of pregnant women admitted to using marijuana. CBD is sometimes used as a remedy to morning sickness and anxiety during pregnancy. The FDA, researchers and physicians strongly advise against using CBD, Δ9-THC, and marijuana in any form during pregnancy. The U.S. Surgeon General has also advised against using marijuana during pregnancy. Meconium, the first stool sample passed after birth, is the specimen of choice to evaluate fetal exposure to marijuana and/or CBD, as it begins to form in the fetal gestational track during the second trimester.

Objective: To develop a method for the analysis of relevant cannabinoids in meconium.

Methods: An ultra performance liquid chromatography–tandem mass spectrometry (UPLC–MS/MS) assay was developed for simultaneous identification of 11 cannabinoids, including Δ9-THC), 11-nor-9-carboxy-Δ9-THC (Δ9-THC-COOH), 11-hydroxy-Δ9 -THC (11-OH-THC), and CBD and its metabolites (6-OH CBD, 7-OH CBD, and 7-COOH-CBD). Calibrators and control materials were prepared in pooled drug-free meconium. Sample preparation was performed in microcentrifuge tubes, deuterated internal standards were added to 0.25gm meconium followed by 200mcL of water. Cannabinoids were extracted into 1 mL acetonitrile and cleaned up using UCT THC FASt® column. Cannabinoids were analyzed using Waters AcQuity Xevo TQS-micro UPLC-MS/MS. Chromatographic separation was performed using an Acquity BEH C18 (50mmx2.1mmx1.7μm) column. The mobile phase was: (A) 20mm ammonium formate in water and (B) 20mm ammonium formate in methanol. The gradient was 0.0 to 5 minutes at 40:60 (A:B respective percentages), 5 to 7 minutes at 0:100, 7 to 7.5 minutes at 0:100 at 7.6 minutes returned to 40:60. Flow rate was 0.5mL/minute and column temperature was 40°C. Ions were collected in Multiple Reaction Monitoring (MRM). Total runtime was 8 minutes.

Method Validation: The method was validated using ASB/ANSI 036 guidelines as a basis for linearity, accuracy and precision, matrix effect, specificity, stability, carryover and limit of detection studies using meconium. Calibrators and controls were prepared at 20-1000 ng/g and 20, 60, 150, 750, and 1000 ng/g respectively. Twelve authentic meconium specimens submitted for Δ9-THC-COOH confirmation were analyzed.

Results: Calibration curves were determined to be linear (r^2=0.999). The lower limit of detection (LLOD) was administratively set at 10 ng/g. Bias was within ± 20%; intra and inter-run precision had CVs ≤ 20%. All analytes were stable under 3 freeze-thaw cycles, bench top stability and 72 hr post preparation conditions, except 6-OH CBD and 7-COOH CBD (stable for 24 hours post preparation). No interfering substances, including several related cannabinoid/hemp compounds (Δ8-THC, CBC, CBG, CBN, and CBL and THCV) nor carryover, were observed. Average recovery was 70% for all analytes. All 12 authentic specimens were negative for 6-OH CBD and 7-COOH-CBD; nine were positive for 7-OH CBD (30-295ng/g); three CBD (14-86ng/g); six CBN (11-72 ng/g); one for Δ9-THC (5 ng/g); six for Δ9-THC-COOH (11-95ng/g) and one for Δ9-THC (5ng/g) and(11-OH-THC (11ng/g).

Conclusion/Discussion: The method was robust and reliable for the quantitation of 11 cannabinoids in meconium. Authentic meconium specimens were determined to contain THC, CBD and their metabolites. This demonstrates that additional cannabinoid testing in meconium specimens may be warranted.

PLATFORM

S-045: LC/QTOF-MS Analysis of Blood and Oral Fluid Submissions in DUID Cases

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Background/Introduction: Oral fluid has gained considerable interest as an additional matrix type to analyze in suspected driving under the influence of drugs (DUID) cases. When collected roadside, oral fluid provides a biological specimen that is much closer to the time of actual motor vehicle operation by an individual. Oral fluid and blood samples are routinely collected as part of DUID investigations in the State of Alabama. The Alabama Department of Forensic Sciences (ADFS) toxicology lab traditionally screens blood and oral fluid case samples using enzyme immunoassay (EIA) technologies. Noticeable drawbacks of EIA include the lack of specificity provided as well as the overall lack of novel psychoactive compounds (NPS) encompassed by the assay that are not included in the traditional EIA "kits", and therefore are missed by this approach. The use of a screening methodology involving liquid chromatography coupled with a quadrupole-time-of-flight mass spectrometry (LC/QTOF-MS) was investigated to expand the analytical scope of analysis beyond the traditional combination of EIA screening with targeted mass spectrometric confirmation.

Objectives: To develop a method utilizing an Agilent 6545 Q-TOF instrument for the analysis of oral fluid and blood specimens as part of DUID casework.

Methods: Blood and oral fluid samples were extracted using a liquid-liquid extraction procedure employing alkalized n-butyl chloride, before dry-down and reconstitution in a 95/5 mixture of mobile phases A and B. The analytical separation of the extracts was performed on an Agilent Poroshell 120 EC-C18 (3 x 100 mm, 2.7 micron) column held at 55° C with mobile phase A consisting of water plus 0.1% formic acid and mobile phase B consisting of acetonitrile with 0.1% formic acid. The MS^2 data were collected in a single analysis at several different collision energies. The results were processed using Agilent MassHunter Qualitative Analysis B.07.00 software and compounds were identified using an in-house compound database and library that included analyte retention times.

Results: Method validation was performed in accordance with SWGTOX guidelines and included the parameters: limit of detection, stability, carryover, interferences, and ion enhancement and suppression. Parameters for acceptance criteria were evaluated and optimized based on a scoring algorithm of the extracted ion chromatograms that included mass accuracy, retention time, isotopic spacing, and isotopic abundance. Mass Hunter Qualitative Analysis software fragmentation data criteria were based on mass accuracy, fragment coelution scoring, and number of qualified fragments. All target ions, precursor and fragments, were identified with a mass tolerance of ±5 ppm. The method was successfully used to analyze blood and oral fluid samples from thirteen specific DUID cases, including six cases containing both matrices. The method has been used since May 2021 to present. Several of these cases identified compounds in both blood and oral fluid that were not included in the list of the "targeted" confirmation methods at ADFS, including novel benzodiazepines, such as flubromazolam.

Conclusion/Discussion: This work demonstrates the application of an Agilent 6545 QTOF for both accurate mass screening and fragment confirmation during the analysis of oral fluid and whole blood case specimens. MS/MS experiments allowed for confirmation of the accurate mass MS results by providing and higher degree of identification confidence. Per the OSAC *Standard for Identification Criteria in Forensic Toxicology* (currently in draft), methods, such as described here, utilizing LC-MS TOF with full scan spectral library matches and TOF-fragmentation provide a high degree of selectivity and as such, earn 4.5 and 7 "identification points," respectively.

S-046: Tianeptine: Antidepressant or Novel Psychoactive Substance (NPS)?

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Background/Introduction: Tianeptine is an atypical antidepressant that enhances, rather than inhibits, serotonin reuptake in the brain. It is available in many countries throughout the world but is not prescribed in the USA. The drug has been shown to have agonist activity at mu and delta opioid receptors making it a candidate for abuse. In the US, tianeptine is being sold on the internet as a supplement and anxiolytic. It is currently a Schedule II controlled substance in certain states but not controlled Federally.

Objectives: In this communication we report on 32 postmortem and 1 driving under the influence (DUI) case involving tianeptine collected over 39 months ending 3/31/21.

Methods: Tianeptine is detected as a non-target finding using accurate mass screening with confirmation by liquid chromatography tandem mass spectrometry (Xevo TQ-XS). Confirmation testing employed solid phase extraction, three levels of standard addition, and utilized positive electrospray ionization and multiple reaction monitoring. Precursor and product ions utilized were: tianeptine – 437.3 > 291.8 (quantification ion), 437.3 > 227.8 (qualifier); tianeptine-D6 – 443.3 > 291.8 (quantification ion), 443.3 > 227.8 (qualifier). The limit of detection was 20 ng/mL. Specimens exceeding the highest calibrator (2000 ng/mL) were diluted. Tianeptine concentrations were reported to 2 significant figures.

Results: The mean and median concentrations of tianeptine in the 33 positive cases were 5362 and 3330 ng/mL, respectively. Concentrations (standard deviation) ranged from 43 – 21000 (5277) ng/mL distributed as noted in the Table 1. Previous cases associated with tianeptine-related deaths had concentrations exceeding 2000 ng/mL; here, 23 (70%) of cases exceeded 2000 ng/mL. Positive cases were separated into 4 cohorts as shown in Table 2.

Table 1

Tianeptine (ng/mL)	N	%
20-1000	4	12.1
1001-2000	6	18.2
2001-5000	12	36.4
5001-10000	5	15.1
>10000	6	18.2

Table 2

Cohort	N	Tianeptine (Mean ng/mL)
Tianeptine Directed (no other tox)	6	5433
With NPS and Other Drugs	10	2744
With Other Drugs (No NPS)	14	5275
Tianeptine Only	3	13433

For the first cohort, testing was for tianeptine only; other drugs present were unknown. The second cohort included cases with both NPS and other substances. Mitragynine was the most frequently detected NPS (N=8, mean concentration: 59 ng/mL) followed by Novel Benzodiazepines (N=4) and one case with valeryl fentanyl. Some cases had multiple NPS drug classes. Other drug classes observed with this group include stimulants and opioids (N=5, each), benzodiazepines (N=4), and gabapentin (N=2). The third cohort included cases with other drugs (without NPS). Frequently observed drugs in this group were benzodiazepines and antidepressants/antipsychotics (N=9, each), gabapentin (N=5), muscle relaxants and/or stimulants (N=3, each) and opioids (N=2). The highest mean tianeptine concentrations occurred in cases where tianeptine was the only drug detected; the lowest mean concentration occurred in cases with additional NPS and other drugs. Cannabinoids (N=6) and ethanol (N=4) were not included above. For the single DUI case tianeptine was present at 1600 ng/mL along with methamphetamine (380 ng/mL), amphetamine (170 ng/mL) and mitragynine (11 ng/mL).

Conclusion/Discussion: Tianeptine-positive cases (33) were separated into 4 cohorts. Of the 27 cases where complete toxicology was performed tianeptine was often present with NPS (37%) and other drugs (52%). In 19 of these cases (70%), tianeptine concentrations exceeded 2000 ng/mL, consistent with a tianeptine-related fatality. As tianeptine is not prescribed in the US, its detection with other NPS suggest that it was obtained like other NPS and could be considered as such. Additionally, its presence with prescribed antidepressants and/or antipsychotics in 10 cases is consistent with the internet marketing of the drug as an anxiolytic.

S-047: Pharmacokinetic Profile of Δ9-Tetrahydrocannabinol (THC), Cannabidiol (CBD), and Metabolites in Whole Blood Following Vaporization and Oral Ingestion of CBD Products.

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Background/Introduction: Cannabidiol (CBD) is widely available for consumption in a variety of preparations and via multiple routes of administration. Few controlled studies have evaluated the pharmacokinetics of CBD in controlled human laboratory studies, especially those in which product formulation or route of administration were manipulated. Some *in vitro* studies suggest that CBD may convert to Δ9-tetrahydrocannabinol (THC) in the gut. Also, the recent federal legalization of hemp allows retail products to legally contain low concentrations of THC ($\leq$ 0.3% THC). The impact of these products on drug testing outcomes has been understudied.

Objectives: We sought to determine 1) whether THC or its metabolites are detected in whole blood after acute administration of 100mg oral or vaporized CBD, and 2) to determine the concentrations of CBD, THC and their metabolites after a single acute administration of vaporized high-CBD (10.5%) cannabis that also contained a low concentration of THC (0.39%).

Methods: Participants (n=18) completed a within-subject, double-blind, double-dummy, placebo-controlled laboratory study. Each participant was exposed, in a within-subject crossover, to placebo, 100mg oral CBD, 100mg vaporized CBD, and vaporized whole plant cannabis that contained 100mg CBD and 3.7mg THC, in randomized order. In the 100mg oral CBD condition, each participant received one of the following three formulations (*n*=6 participants/formulation): (1) encapsulated CBD (2) CBD suspended in pharmacy-grade cherry syrup, or (3) Epidiolex® (the FDA-approved and marketed CBD product). Six participants completed a 5th test session in which 100mg oral CBD was administered after overnight fasting. Whole blood samples were collected immediately before and periodically for three days after drug administration. Immunoassay screening for THC-COOH and LC-MS-MS confirmatory tests CBD, CBN, Δ8- and Δ9-THC, and select metabolites were performed on each biospecimen collected.

Results: Blood concentrations of CBD were greatest after vaporized CBD-dominant cannabis (mean Cmax: 171ng/mL, range: 40-665ng/mL) versus vaporized pure CBD (mean Cmax: 105ng/mL, range: 19-312ng/mL) and oral pure CBD (collapsed across oral formulation, mean Cmax: 14ng/mL, range: 0-50ng/mL). Of the three oral formulations, Epidiolex produced the highest peak concentration of CBD relative to encapsulated CBD and CBD in syrup (Epidiolex mean Cmax = 21ng/mL, range: 8-37ng/mL; encapsulated CBD mean Cmax = 18, range: 2-50; CBD in syrup mean Cmax = 3, range: 0-7). CBD concentrations were lower and had a shorter window of detection when administered after overnight fasting compared with administration with a low-fat breakfast. THC was detected in the blood of 12/18 participants after vaporized high-CBD cannabis (mean Cmax: 5ng/mL, range: 0-31ng/mL), but was not detected in the blood of participants after vaporized or oral CBD administration.

Conclusion/Discussion: Analysis of whole blood showed no indication that oral CBD converts to THC or its metabolites in the human gut after acute administration. There were clear differences in bioavailability by both route of administration, product formulation, and gastric contents. Inhalation of vaporized CBD-dominant cannabis containing 0.39% THC produced blood levels of THC in a subset of participants that exceed *per se* cut-offs commonly used by states to identify individuals believed to be intoxicated from cannabis use. Additional research is needed to characterize whole blood cannabinoid concentrations after acute and chronic use of a variety of legal hemp products that contain greater than 0, but less than or equal to 0.3% THC to help interpret drug testing outcomes.

S-048: Xylazine Prevalence in a Chronic Pain and Behavioral Health Population

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Background/Introduction: Xylazine (Rompun®) is a sedative and α2 adrenergic receptor agonist that is used in conjunction with ketamine as an anesthetic in veterinary medicine. Its sedative properties and more widespread availability make xylazine a desirable choice for illicit substance adulteration and direct abuse has been documented in post- and ante-mortem toxicology reports, either alone or in combination with fentanyl, heroin, or cocaine. Xylazine intake (intentional or unintentional) has been observed more frequently in the last 10 years and was recently reported as detected in >30% of Philadelphia fentanyl and/or heroin overdose fatalities in 2019. At-risk patients under treatment for chronic pain, opioid use disorder, or substance use disorder often struggle with addiction to illicit substances; little information exists on the detection of xylazine in this population. As such, we evaluated a cohort of samples from our patient population to provide the clinical and forensic toxicology communities a better understanding of the prevalence of xylazine consumption.

Objectives: Upon attending the presentation, the attendee will be able to describe the prevalence of urine xylazine in chronic pain and behavioral health populations. This presentation will guide the learner to consider xylazine as a potential adulterant and/or intoxicant when conducting forensic or clinical evaluations.

Methods: This study was IRB approved. Xylazine was analyzed in the laboratory as part of a larger Novel Psychoactive Substances (NPS) panel for which target analytes were chosen based on information from the US Drug Enforcement Administration as well as online resources. Prior to analysis, analytes were extracted from hydrolyzed urine using a liquid-liquid extraction followed by evaporation and reconstitution in mobile phase. Samples (10 µL) were injected onto a liquid chromatography/tandem mass spectrometry (LC-MS/MS) instrument consisting of a Shimadzu Prominence HPLC and Sciex API 4000 MS/MS. The mass spectrometer was operated in positive electrospray ionization mode for scheduled multireaction monitoring (sMRM) analysis (two transitions per analyte). Analytes were chromatographically separated on a biphenyl column with formic acid (0.1%) modified water/acetonitrile. Upon request by a provider, samples were analyzed for any NPS compound (or drug class) during the study period of April 5 - May 5, 2021, and all samples meeting qualitative acceptance criteria for xylazine were included in this analysis.

Results: Of 21,421 samples evaluated, 184 (0.9%) contained detectable xylazine. Samples were limited to those submitted for NPS testing. In samples submitted, the largest rates (N) of detection were observed in Kentucky (51), Louisiana (33), and Maine (29). Ages (N, %) and gender distributions were: 18-25 years (2, 1%), 26-40 years (130, 71%), 41-56 years (35, 19%), and 57-75 years (17, 9%) with 73 (39.6%) female and 110 (60%) male samples; one sample was not specified. Of the positive samples, 167 (91%) were also positive for fentanyl. Of these samples co-positive for fentanyl, 14 samples were also positive for heroin markers, while 11 others were positive for cocaine and heroin as well. Only 1 sample was observed to be positive not in the presence of illicit substances, or buprenorphine/methadone.

Conclusion/Discussion: Xylazine is a potent veterinary sedative and known illicit drug adulterant that has increased in detection in recent years. While the effects of xylazine in humans is still not fully understood, particularly in combination with illicit drugs, these prevalence data raise awareness of a potential co-ingested NPS and adulterant that can assist clinicians and toxicologists in treatment decisions and case determinations.

PLATFORM

S-049: Urinary pharmacokinetics of immediate and controlled release oxycodone and its phase I and II metabolites using LC-MS/MS

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Background/Introduction: Oxycodone is a schedule II semi-synthetic opioid in the United States that is prescribed for its analgesic effects and has a high potential for abuse. Prescriptions for oxycodone can vary by dose and its formulation, immediate release (IR) and controlled release (CR). Monitoring metabolites of oxycodone can be beneficial for forensic casework. However, the few studies involving time resolved pharmacokinetics of the urinary excretion of oxycodone metabolites leave a knowledge gap regarding the elimination of conjugated and minor metabolites, pharmacokinetic differences by formulation, and the impact of CYP2D6 activity on the metabolism and excretion of oxycodone.

Objectives: This study sought to compare urinary excretion of phase I and II metabolites by formulation and compare ratio changes over time by assessing metabolic ratios, formulations, and predicted phenotype.

Methods: Subjects (n=7) received a single 10 mg IR tablet of Oxycodone Actavis. A few weeks later, the same subjects received a single 10 mg CR tablet of Oxycodone Actavis. During each setting, urine was collected at 0, 0.5, 1, 1.5, 2, 3, 4, 5, 6, 8, 9, 10, 12, 14, 24, 48, and 72 h. Urine samples (100 μL) were diluted with 900 μL water containing internal standards and analyzed on an Acquity UPLC® I-class coupled to a Waters Xevo TQD using a previously validated method [1]. Separation was achieved using an Acquity HSS T3 column (1.7 μm, 2.1 x 100 mm) at 30°C at 0.5 mL/min. Mobile phase A was 0.001% formic acid in 10 mM ammonium formate (pH 5.2) and mobile phase B was 0.001% formic acid in acetonitrile. The limits of quantification were 0.015 μg/mL for oxycodone, noroxycodone, 6α/β-oxycodol, oxymorphone, and noroxymorphone and 0.050 μg/mL for noroxymorphone-3ß-D-glucuronide and oxymorphone-3ß-D-glucuronide. Creatinine (mg/mL) and oxycodone screening were performed on an AU680. Urine drug concentrations were creatinine-normalized (μg/mg). The CYP2D6 phenotypes were categorized as poor metabolizers (PM), intermediate metabolizers (IM), extensive metabolizers (EM), and ultra-rapid metabolizers (UM). Comparisons between IR and CR were performed using two-tailed paired t-test at a significance level of p=0.05.

Results: Analytes were grouped into major (oxycodone, noroxycodone, oxymorphone-3ß-D-glucuronide) and minor analytes (6α/β-oxycodol, oxymorphone, noroxymorphone-3ß-D-glucuronide) and phenotypes were grouped (IM/EM and PM) for the comparisons. When comparing the formulations, mean oxycodone C_{max} after IR dose was significantly higher (3.4 vs. 1.3 μg/mg), detected significantly earlier (0.93 h for IR and 1.2 h for CR) and reached maximum concentration earlier (2.1 h for IR and 5.1 h for CR) compared to CR. Noroxycodone t_{max} after IR was significantly earlier (3.1 h for IR and 7.4 h for CR) and detected significantly longer (48 h for IR and 65 h for CR) than CR. Oxymorphone-3ß-D-glucuronide reached maximum concentration significantly earlier (3.7 h for IR and 8.0 h for CR) and was detected significantly longer (48 h for IR and 65 h for CR) after IR compared to CR. The following metabolite ratios were evaluated with noroxycodone/oxycodone and oxymorphone-3ß-D-glucuronide/oxycodone shown below in Figure 1.
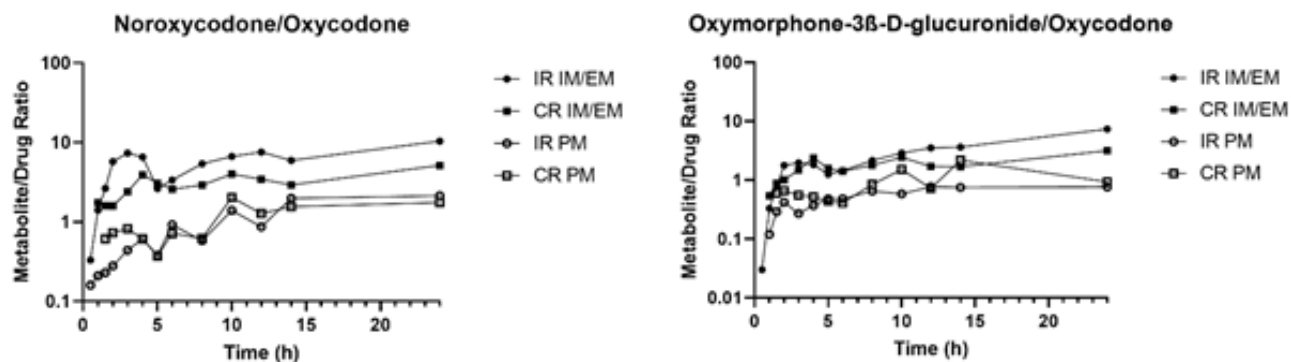
Figure 1. Metabolite to drug ratios over time following administration

Conclusion/Discussion: The inclusion of noroxycodone and phase II metabolites (especially oxymorphone-3ß-D-glucuronide) increased the detection window of oxycodone use. The metabolite ratios showed a general increase over time. Differences were observed depending on the phenotype of the subject. Metabolite/drug ratios for PM were lower after both IR and CR compared to IM/EM. Understanding these differences is necessary if ratios are used to estimate time of intake of an individual.

Reference: Truver M.T., Jakobsson G., Cherma M.D., Swortwood M.J., Green H., and Kronstrand R. (2020) The quantification of oxycodone and its phase I and II metabolites in urine. Journal of Analytical Toxicology, https://doi.org/10.1093/jat/bkaa186.

S-050: A Case Series Of N-ethylhexedrone Intoxications

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Background/Introduction: We present here a clinical case series of three N-ethylhexedrone (Hexen, NEH) intoxications documented with blood and urine concentrations. A thorough investigation of all the metabolites produced was carried out. Two of the three patients had multiple samples (blood and urine) taken during the days following their arrival at the hospital. The last patient only had 1 blood and 1 urine sample.

Objectives: NEH is a synthetic cathinone whose use spread into Europe during second part of the last decade. Most of what we know about this drug comes from harm reduction websites and forums. Presentation of these three clinical cases will allow a better understanding of the effects (expected or adverse reactions) and elimination of this drug.

Methods: NEH was identified in all samples with LC-HR/MS after precipitation (UPLC-ESI$^+$-Q-TOF, MS^E mode) and then quantified with LC-MS/MS after liquid extraction (MRM mode). Potential metabolites have been identified by means of LC-HR/MS using the same mode and the software Metabolynx^{TM} (Waters, Milford, MA, USA).

Results: The three patients were men aged 26 to 33 years. The first case was a man who was found unconscious by his roommate and was still confused after awakening. He was hospitalized in the psychiatric unit for 11 days, during which he had 5 blood samples and 4 urine samples taken. The first blood sample revealed the presence of NEH at screening. Quantification occurred later and showed concentrations ranging from 49 (Day 1) to 1.8 (D7) ng/mL in blood. Four metabolites were identified in blood and urine (NEH + H_2, NEH + O, NEH – C_2H_4 and NEH – C_2H_4 + O). The second case was a man with a history of drug use (drugs bought on the internet). He was found drowsy in the bathroom by his father, and then fell into a coma upon arrival to the emergency unit. After one day in the intensive care unit he regained consciousness, was aggressive and unwilling to be treated. He left the hospital without discharge. A concentration of 7.9 ng/mL NEH was found in blood sampled at arrival. Another cathinone of the chloromethcathinone series and etizolam were also identified. The last patient was found unconscious with seizure by his roommate. At arrival in the emergency department, major hyponatremia and lactic acidosis was noted; a syndrome of inappropriate antidiuretic hormone secretion (SIADH) was diagnosed. He stayed at the hospital for 7 days, during which samples of blood and urine were taken every day. Concentrations of NEH ranged between 97.2 (D1) and 0.7 (D7) ng/mL for blood and between 724 (D1) and 0.5 (D7) ng/mL for urine.

Conclusion/Discussion: The cases presented highlight the long elimination half-life of NEH. Four metabolites have been identified which could further allow a better identification of the consumption of this drug. Serious adverse effects can be observed after use of NEH, 2 of the 3 patients required intubation and ventilation. A SIADH syndrome was also diagnosed with similarities to what is frequently seen with MDMA intoxication. Two of the three cases are noteworthy because of the number of samples taken, and also by the fact that NEH was the only drug of abuse detected.

PLATFORM

P-001: SAMHSA Drug Panel Screening in Oral fluid: Development of screening method at 8 seconds per sample using LDTD-MS/MS.

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Background/Introduction: In 2019, the US Department of Health and Human Services (via the SAMHSA agency) established scientific and technical guidelines for federal workplace drug testing programs in oral fluids (Federal Register / Vol. 84, No. 207, 2019). Screening various drug classes requires several different immunoassay reagents or an LC-MS/MS method with a longer analysis time per sample. LDTD-MS/MS technology combines the speed and the analysis of different drug classes within a single method.

Objectives: The goal of this presentation is to use an automated sample preparation method for LDTD-MS/MS screening of all compounds in a single operation. Two types of collection devices for oral fluid were evaluated: Intercept I2™ device and Oral-Eze® device.

Methods: Drug-free oral fluids of different volunteers were collected using the Intercept I2™ and Oral-Eze® device. After the collection of the oral fluid, the pad was transferred into a tube containing an extraction buffer. During this process, oral fluids are diluted by a factor of 3. The SAMHSA drug panel for an oral fluid screen was spiked in both extracts at a concentration around the decision point cut-off. Samples were extracted using the Azeo automated extraction system. The robot scans the barcode of the sample, generates a batch file for the LDTD-MS/MS system, extracts the sample and spots the LazWell plate. The extraction buffers of the collection devices have different chemical constituents and need a specific sample preparation. For the Intercept I2™ device, a salt assisted liquid-liquid extraction (SALLE) was used, and for the Oral-Eze® device, a sample dilution approach was set. Positive ion mode was utilized for the following drugs: amphetamine, methamphetamine, MDA, MDMA, PCP, morphine, hydromorphone, codeine, hydrocodone, cocaine, oxymorphone, oxycodone and 6-MAM. The negative ionization mode was used for THC. The LDTD-MS/MS source operated in MRM mode with polarity switching on Sciex Q-Trap 5500 mass spectrometer allowed for rapid detection (8 seconds per sample) of all drugs desorbed simultaneously for screening. Specific transitions were monitored for each drug to screen samples and cutoff standard. LUXON ion source model was used as LDTD system with a laser ramps de 3 seconds to 55% and hold of 2 seconds with a carrier gas (Air) flow rate of 3 L/min.

Results: For the method validation, negative saliva sample was spiked at 0.5X, 1X and 2X of the cutoff concentration defines by SAMHSA. Spiked samples and blank solutions were used to validate the precision of the method. The peak area against the internal standard ratio was used to normalize the signal. For all drugs, no overlapping at the cutoff decision point is observed and the %CV was between 1.8% to 16.7% for inter-run experiments. Oral fluids were collected from ten different volunteers. Samples were screened to verify the presence of each analyte (all samples were negative). Drugs were spiked at 50% cut-off (QC-L) and 200% cut-off (QC-H) and screened as unknown for the cross-validation study. The LDTD-MS/MS results were used to evaluate the following validation parameters: the method sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and accuracy. A percentage of 100% was obtained for all validation parameters of all drugs.

Conclusion/Discussion: Luxon Ion Source® combined to a Sciex Q-Trap 5500 mass spectrometer system allows ultra-fast (8 seconds per sample) screening of a SAMHSA drug panel in oral fluid using a simple and automated sample preparation method.

POSTER

P-002: High-Throughput Screening Method in Hair Sample: Development of a Screening Method at 8 seconds per Sample using LDTD-MS/MS

Serge Auger*, Sandra Imrazene, Jean Lacoursière and Pierre Picard

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Background/Introduction: Because the hair root is vascularized during growth, illicit drugs present in the blood stream can enter the hair shaft via the root where they will be sequestered; allowing them to be analyzed in small samples of hair. To increase the analysis throughput of hair samples, the LDTD Ion Source® coupled to tandem mass spectrometry (MS/MS) was used for the identification and quantification of drugs of abuse.

Objectives: For this project, we propose to perform a generic extraction method for illicit drug analysis in hair. Screening using the LDTD (Laser Diode Thermal Desorption) coupled to a mass spectrometer (LDTD-MS/MS) is chosen as a fast-analytical technique.

Methods: A pre-wash of the hair is performed to remove external contaminants using methanol. Hair (10 mg) was cut into small pieces (1-3 mm) are transferred in a vial. Two mL of methanol containing TFA at 0.5% (with internal standard (IS): Amphetamine-d11, Methamphetamine-d11, MDMA-d5, Diethylpropion-d10, Mazindol-d4, Morphine-d6, Codeine-d6, Cocaine-d3, 6-MAM-d6 and THC-d3) is added and samples are soaked at 60 °C for 105 minutes. Samples are then sonicated for 15 minutes. After the extraction, 4 µL KH_2PO_4 (100 µM) in water followed by 4 µL of extracted sample are spotted into 96-LazWell™ plates and evaporated to dryness at 40 °C for 8 minutes. LDTD-MS/MS analysis is done after a complete evaporation. Positive ion mode was utilized for the following drugs: Amphetamine, Methamphetamine, MDA, MDMA, MDEA, Diethylpropion, Mazindol, Morphine, Codeine, Cocaine and 6-MAM. The negative ionization mode was used for THC. The LDTD-MS/MS source operated in MRM mode with polarity switching on Sciex Q-Trap 5500 mass spectrometer allowed for rapid detection (8 seconds per sample) of all drugs desorbed simultaneously for screening. Specific transitions were monitored for each drug to screen samples and cutoff standard. LUXON ion source model was used as LDTD system with a laser ramps de 3 seconds to 55% and hold of 2 seconds with a carrier gas (Air) flow rate of 6 L/min.

Results: A screening cutoff concentration of 200 pg/mg hair was used for: Amphetamine, Methamphetamine, MDA, MDMA, MDEA, Diethylpropion, Mazindol, Morphine, Codeine and 6-MAM. Screening decision points of 500 pg/mg Hair and 50 pg/mg Hair were used for Cocaine and THC, respectively. For the method validation, negative hair was spiked at 0.5X, 1X and 2X of the cutoff decision point concentration. Spiked samples and blank solutions were used to validate the precision of the method. The peak area against the IS ratio was used to normalize the signal. For all drugs, no overlapping at the decision point is observed and the %CV was between 2.9% to 17.3% for inter-run experiments. Real hair samples (n=34) were extracted and screened with the LUXON-MS/MS method. The same sample extracts were also analyzed with an LC-MS/MS method as a reference method for a cross-validation study. The LDTD-MS/MS and LC-MS/MS results were used to evaluate the following validation parameters: the method sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and accuracy. A percentage of 100% was obtained for all validation parameters of all drugs.

Conclusion/Discussion: Luxon Ion Source® combined to Q-Trap 5500 mass spectrometer system allows ultra-fast (8 seconds per sample) screening of drugs in hair sample using a generic sample preparation.

P-003: Detection of Δ9-Tetrahydrocannabinol and Metabolites in the Meibomian Lipids of Tear Samples Through LC-MS/MS

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Background/Introduction: This research focused on the detection and quantitation of THC, 11-Hydroxy- THC (11-OH-THC), and 11-nor-carboxy-THC (THCOOH) as these analytes are produced in the metabolism of Δ9-THC. Meibomian fluid maintains a high lipid concentration and Fatty Acid Binding Protein 5 (FAPB5), a protein known to bind to cannabinoids. Due to the lipophilic nature of THC, tear fluid could be used as a less-invasive biological matrix to test for the presence of THC and its metabolites.

Objectives: There exist limitations with current methods of detection of Δ9-Tetrahydrocannabinol (THC) drug analyte in Driving Under the Influence of Drugs (DUID) cases. This research explores the use of meibomian tear fluid as a novel matrix to detect THC and its accompanying analytes.

Methods: This project optimized a collection method for tear fluid that would be more suitable for the creation of a novel direct injection method. Collection was completed by BVI Weck-Cel Sterile Cellulose strips, measuring approximately 2 x 20 mm, and placed in Thompson eXtreme PVDF 0.2 mm, pre-slit, red cap, filter vials containing Quantisal buffer solution. All analysis and calibrations were completed with fortified matrix standards with concentrations ranging from 0.25 - 250 ng/mL. Method validation was consistent with the Academy Standards Board (ASB) Standards of Forensic Toxicology Standard 036, First Edition 2018. Tear samples were collected from four participants according to Institutional Review Board (IRB) standards before and after administration of Marijuana. Samples were collected approximately 30 minutes post administration of THC. Samples and calibration standards were analyzed using Liquid Chromatography Tandem Mass Spectrometry (LC/MS-MS) with the QSight® 220 CR LC-MS/MS and using a Halo® C18 3.0x50 mm (2.7 μm) column.

Results: Limit of Detection (LOD) and Quantitation (LOQ) for THC was calculated at 0.25 ng/mL. The LOD of THCOOH was detected at 0.25 ng/mL and LOQ was calculated at 1 ng/mL. The LOD of 11-OH-THC was detected at 2 ng/mL and was not quantitated. The charted retention times vs analyte intensities were evaluated for the existence of peaks within the expected range. This expected range came from the calibration data as well as internal standard comparison. The goal was to observe peaks around the expected retention time for detection and to observe the area of those peaks to gather quantitative data. The data was then plotted on the calibration curves in order to perform quantitative analysis. As can be seen in Figures 1 and 2 the procedure was sufficient in detection of THC and THCCOOH. Detection of 11-OH-THC was not sufficient. Data was referenced against blood samples taken at the same time as the tear samples (seen in Table 1).
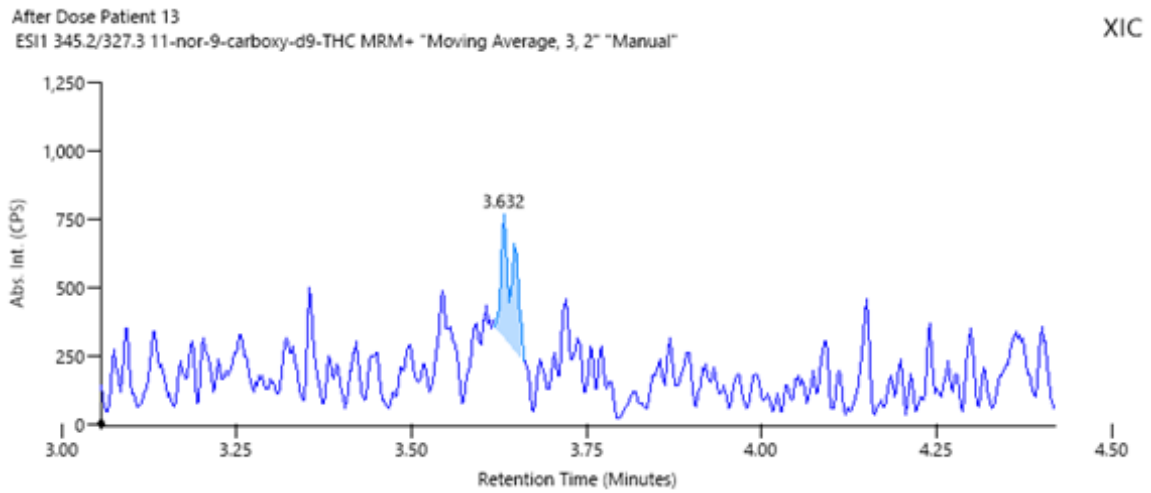
Conclusion/Discussion: Upon analysis of participant samples, it was determined that THC and metabolites could be detected and quantitated in tear fluid. However, it is noted that insufficient sample volume in collection is an issue that leads to poor quantitation and can readily be optimized in future research.

POSTER

Figure 1: Data Collected After Dose Patient 13 - THC

After Dose Patient 13
ESI1 315.3/123.3 d9-THC MRM+ "Moving Average, 3, 2" "Manual"

XIC

MANUA
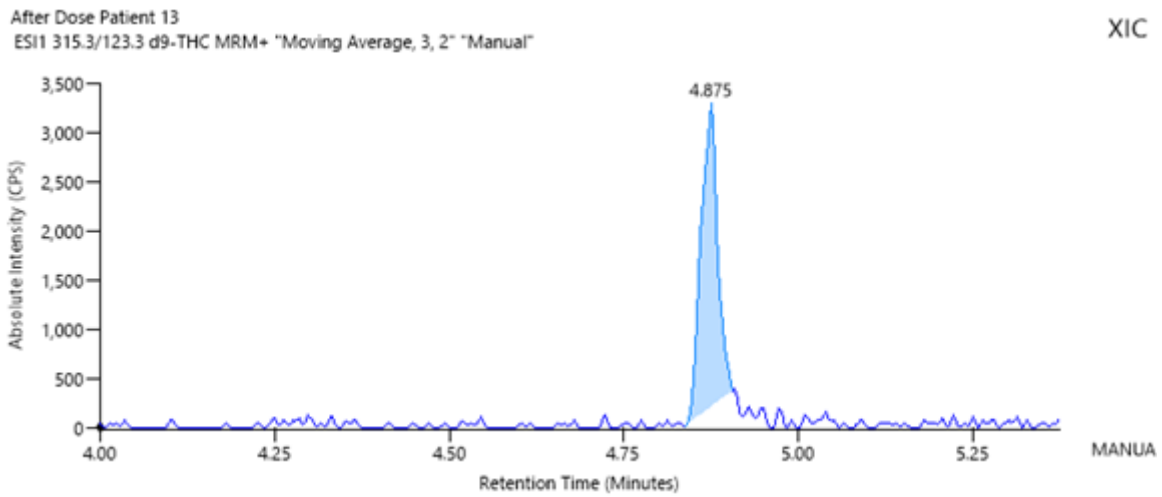
Figure 2: Data Collected After Dose Patient 13 – THCCOOH

After Dose Patient 13
ESI1 345.2/327.3 11-nor-9-carboxy-d9-THC MRM+ "Moving Average, 3, 2" "Manual"

XIC

Table 1: Blood Cannabinoid Concentration Results – Patient 13

Collected Matrix		Concentration ng/mL	Lab Cutoffs ng/mL
Blood	THC	31	0.50
	11-OH-THC	5.8	1.0
	THCOOH	54	5.0

P-004: Semi-quantitative multiplex screening of multiple drugs from a single urine sample in less than 30 minutes on the biochip analyser Evidence MultiSTAT

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Background/Introduction: Drug detection involves initial screening of samples for drugs. Biochip array technology enables the detection of multiple drugs from a single sample by incorporating simultaneous immunoassays on the biochip surface, which define discrete test regions (DTRs). Such a multi-analytical approach increases the screening capacity during the drug testing process. By applying the immunoassays to the fully automated benchtop biochip analyser Evidence MultiSTAT the speed of the analysis is increased.

Objectives: This study reports the application of this system to the simultaneous semi-quantitative detection of drugs of abuse and prescription drugs (creatinine included) in urine in less than 30 minutes; the assays of interest and the cut-offs can be selected, and two samples can be analysed in parallel. This application will facilitate the screening process in testing laboratories.

Methods: Simultaneous competitive chemiluminescent immunoassays (n=28), defining discrete test sites on the biochip surface, were employed. The assays were applied to the Evidence MultiSTAT biochip analyser, which processes a self-contained cartridge comprising all the components required for the assays and the system software processes and reports the multiple semi-quantitative results generated.

Results: Analytical evaluation of the simultaneous semi-quantitative detection of drugs in urine on the Evidence MultiSTAT.

Assay	Standardising Antigen	Min Cut Off Concentration	Max Cut Off Concentration	Sensitivity	Inter Assay Precision	Recovery
Acetaminophen	Acetaminophen	N/A	50µg/ml	≤12.5µg/ml	≤20%	70-130%
Amphetamine	S(+)-Amphetamine	200ng/ml	1000ng/ml	≤50ng/ml	≤20%	70-130%
Barbiturates	Phenobarbital	200ng/ml	300ng/ml	≤50ng/ml	≤20%	70-130%
Benzodiazepines	Oxazepam	100ng/ml	300ng/ml	≤25ng/ml	≤20%	70-130%
Buprenorphine	Norbuprenorphine	1ng/ml	10ng/ml	≤0.25ng/ml	≤20%	70-130%
Benzoylecgonine	Benzoylecgonine	100ng/ml	300ng/ml	≤25ng/ml	≤20%	70-130%
Cannabinoids (THC)	(-)-11-nor-9-Carboxy-Δ9-THC	20ng/ml	50ng/ml	≤5ng/ml	≤20%	70-130%
Dextromethorphan	Dextro-methorphan	N/A	20ng/ml	≤5ng/ml	≤20%	70-130%
Ethylglucuronide (EtG)	Ethyl β-D-Glucuronide	N/A	1000ng/ml	≤500ng/ml	≤20%	70-130%
Fentanyl	Fentanyl	1ng/ml	10ng/ml	≤0.25ng/ml	≤20%	70-130%
Haloperidol	Haloperidol	N/A	50ng/ml	≤12.5ng/ml	≤20%	70-130%
Ketamine	Norketamine	100ng/ml	300ng/ml	≤25ng/ml	≤20%	70-130%
MDMA	(±) MDMA	50ng/ml	500ng/ml	≤12.5ng/ml	≤20%	70-130%
Meprobamate	Meprobamate	N/A	500ng/ml	≤125ng/ml	≤20%	70-130%
Methadone	(±) Methadone	200ng/ml	500ng/ml	≤50ng/ml	≤20%	70-130%
Methamphetamine	S(+)-Methamphetamine	200ng/ml	1000ng/ml	≤50ng/ml	≤20%	70-130%
Methaqualone	2'-Hydroxymethyl Methaqualone	200ng/ml	300ng/ml	≤50ng/ml	≤20%	70-130%
Opiates	Morphine	200ng/ml	2000ng/ml	≤50ng/ml	≤20%	70-130%
Oxycodone	Noroxycodone	100ng/ml	500ng/ml	≤25ng/ml	≤20%	70-130%
Phencyclidine (PCP)	Phencyclidine	N/A	25ng/ml	≤6.25ng/ml	≤20%	70-130%
Pregabalin	Pregabalin	1000ng/ml	2000ng/ml	≤250ng/ml	≤20%	70-130%
Propoxyphene	(+) Propoxyphene	N/A	300ng/ml	≤75ng/ml	≤20%	70-130%
Salicylate	Salicylic Acid	N/A	50µg/ml	≤12.5µg/ml	≤20%	70-130%
Tramadol	Tramadol	100ng/ml	300ng/ml	≤25ng/ml	≤20%	70-130%

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Tricyclic antidepres-sants (TCAs)	Nortriptyline	150ng/ml	1000ng/ml	≤37.5ng/ml	≤20%	70-130%
Zolpidem	Zolpidem	N/A	20ng/ml	≤5ng/ml	≤20%	70-130%
6-MAM	6-MAM	N/A	10ng/ml	≤2.5ng/ml	≤20%	70-130%
Creatinine	Creatinine	N/A	20mg/dl	≤5mg/dl	≤20%	70-130%

Conclusion/Discussion: Results show applicability of the simultaneous immunoassays applied to the Evidence MultiSTAT to the rapid semi-quantitative screening of a broad range of drugs from a single sample. User defined cut off concentrations can be selected to suit multiple drugs of abuse screening applications or the cut off requirements per region. The extensive test menu includes tests for fentanyl and tramadol, which present increasing prescription rates (1, 2) and for phencyclidine which is much less often abused but still retained in traditional panels (3). The benzodiazepines assay has a broad specificity profile for the detection of traditional and designer benzodiazepines, making this application a multi-drug flexible screening option for updated drug trends.

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POSTER

P-005: Method Validation for the Identification of Gamma-Hydroxybutyrate (GHB) in Blood, Urine and Vitreous by GC-MS/MS

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Background/Introduction: Gamma-hydroxybutyrate (GHB) is an abused substance in the United States, commonly associated with drug-facilitated sexual assaults due to its fast-acting sedative and amnesic properties. GHB poses analytical and interpretive challenges due to its polar structure, rapid elimination and endogenous presence. In postmortem cases, GHB levels in blood may increase after death; therefore, analyzing alternative matrices that are less susceptible to postmortem changes, such as vitreous humor, may aid in interpretation. GHB is rapidly metabolized and eliminated after ingestion making early testing a critical factor. In our laboratory, GHB was previously analyzed in blood and urine by first acidifying GHB to gamma-butyrolactone (GBL), performing liquid-liquid extraction and measuring the concentration of both GHB+GBL by GC-MS. A more robust and efficient analytical method using newer, more sensitive instrumentation was desired.

Objectives: To validate a quantitative GHB method in blood, urine, and vitreous using solid phase extraction (SPE) and GC-MS/MS.

Methods: GHB and its deuterated internal standard were extracted from 100 μL of blood, urine and vitreous using anion exchange SPE. The analytes were eluted using 94:6 methanol:acetic acid, evaporated to dryness under nitrogen at <40°C, reconstituted with acetonitrile, and derivatized with BSTFA with 1% TMCS for 5 minutes at 70°C. The derivatized samples were analyzed on an Agilent 7000 Series GC-MS/MS with DB-35MS column using a temperature program that started at 65°C was held for 1 min, increased 20°C/min to 130°C, then increased 50°C/min to 250°C with a total run time of 7.65 minutes. Data were collected in multiple reaction monitoring (MRM) mode to collect two transitions for the di-TMS derivatives of GHB and GHB-d6. For quantitation, an aqueous calibration curve and matrix-matched controls were used since GHB is an endogenous compound and a negative blank blood was unavailable. The assay was validated in accordance with ANSI/ANAB Standard 036, as well as the criteria set by American Board of Forensic Toxicology standards for calibration model, bias, precision, dilution integrity, stability, Limit of Detection (LOD), carryover, interferences, and limit of quantitation (LOQ).

Results: A weighted (1/X) linear calibration model of 5-100 mg/L was used to quantitate GHB in blood, urine, and vitreous humor. Bias and precision of matrix-matched controls did not exceed -13% and 10%, respectively. Dilutions of 1:2 or 1:10 were acceptable for quantitative results in blood. Processed samples were stable for up to 9 days. LOD was administratively set to 5 mg/L and all qualitative acceptance criteria were met. There was no carryover observed in negative controls that were analyzed immediately after the highest calibrator. No interferences were observed from internal standard, blank matrices or other common analytes. LOQ was established at the lowest calibrator concentration of 5 mg/L and met bias and precision acceptance criteria.

Conclusion/Discussion: A quantitative method was developed and validated for the analysis of GHB in blood, urine, and vitreous humor. The method utilized a simpler and cleaner SPE with silylation that increased the sensitivity and chromatography, on a more sophisticated GC-MS/MS without the need to convert GHB to GBL prior to extraction. In postmortem toxicology, the analysis of vitreous humor aids in interpretation between exogenous and endogenous levels of GHB.

POSTER

P-006:   Comparison of *In Vitro* Systems for the Generation of Drug Metabolites in Forensic Toxicology

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Background/Introduction: *In vitro* metabolic assays are commonly used in forensic toxicology to assess the extent and rate of drug metabolism and to identify specific metabolites formed. An *in vitro* enzymatic assay requires an analyte of interest and a means of metabolizing that analyte, typically a type of liver fraction such as human liver microsomes (HLM). However, other *in vitro* systems are also available, including synthetic metalloporphyrins that mimic oxidative metabolism by cytochrome P450 and electrochemical oxidation that can mediate reactions such as aromatic hydroxylation, dehydrogenation, and O- and N-dealkylation. These assays may also have utility in identification of reactive metabolites. While enzyme-based assays are widely employed for drug metabolite generation in forensic toxicology, alternative *in vitro* systems have not been extensively tested.

Objectives: The goal of this research was to compare the Phase I metabolites formed by several drugs of interest using three different *in vitro* models: 1) human microsomal enzymatic assays, 2) synthetic metalloporphyrin catalysts, and 3) electrochemical oxidation. A primary focus of the study was to elucidate differences in the stable metabolites generated by each system.

Methods: For the first approach, an *in vitro* enzymatic assay using human liver microsomes and NADPH was applied. Briefly, all assay components and cofactors were combined in a microfuge vial and vortexed, followed by an incubation of 4 h at 37°C. Following centrifugation, the supernatant was transferred to LC vials for analysis. For the synthetic metalloporphyrin catalysts approach, a commercial biomimetic kit (BMO kit; HepatoChem, Inc.) was employed. The kit tests 50 different reaction conditions to find the optimal one for each drug. For electrochemical oxidation, a system of three electrodes was employed: glassy carbon as the working electrode, calomel standard as the reference electrode, and platinum rod as the counter electrode. Bulk electrolysis was performed, where a solution containing 4 mM of drug was oxidized. Analysis of the formed metabolites of acetaminophen (APAP), 3,4-methylenedioxymethamphetamine (MDMA), methamphetamine (METH), and Δ^9-tetrahydrocannabinol (THC) was performed using high resolution MS on an Agilent 1290/6530 LC-QTOF-MS system.

Results: The *in vitro* enzymatic assay demonstrated formation of N-acetyl-p-benzoquinone imine (NAPQI) and 1,4-benzoquinone for APAP; 3,4-methylenedioxyamphetamine (MDA), 3,4-dihydroxymethamphetamine (HHMA), 4-OH-3-formate amphetamine, and an aminochrome intermediate for MDMA; amphetamine, N-hydroxyamphetamine (N-OH-AMP), N-hydroxymethamphetamine (N-OH-METH) and methcathinone for METH; and 11-COOH-THC and 11-OH-THC for THC. Use of synthetic metalloporphyrin catalysts demonstrated formation of NAPQI for APAP; α-methyldopamine (α-MeDA), 4-hydroxy-3-formyl amphetamine, 4-hydroxy-3-methoxyamphetamine (HMA), MDA, and a 4-hydroxy-3-bicarbonate amphetamine derivative for MDMA; amphetamine, *p*-hydroxymethamphetamine (p-OH-METH), and methcathinone for METH; and 11-COOH-THC, 11-OH-THC, 11-oxidocarbonate, and 11-vinyl derivatives for THC. The electrochemical oxidation generated NAPQI for APAP; MDA, 4-hydroxy-3-formyl amphetamine, and a quinone intermediate for MDMA; amphetamine, p-OH-METH, methcathinone, and an amphetamine imine derivative for METH; and 11-COOH-THC, 11-oxidocarbonate, and 11-vinyl derivatives for THC.

Conclusion/Discussion: Metabolites obtained with the three different *in vitro* systems exhibited a few common derivatives but also compounds unique to each system. In addition, the major reported *in vivo* metabolites for each drug were also found with all three *in vitro* systems, *i.e.,* NAPQI, MDA, amphetamine, and 11-COOH-THC. The synthetic metalloporphyrin and electrochemical oxidation systems appeared to generate a wider variety of metabolites than encountered with human liver microsomes, including stable and potentially reactive derivatives. These results indicate that use of all three *in vitro* systems may provide a more complete profile of potential Phase I oxidative metabolites for a variety of drugs that may be targeted for analysis in forensic toxicological studies. Research is continuing to further evaluate the utility and effectiveness of alternative *in vitro* metabolic assays for identifying stable and reactive metabolites for drug screening applications.

POSTER

P-007: The rise and fall of isotonitazene and brorphine: two recent stars in the new synthetic opioid firmament

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Background/Introduction: Synthetic opioids constitute one of the fastest growing groups of new psychoactive substances worldwide. With fentanyl analogues being increasingly controlled via class-wide scheduling, many non-fentanyl related opioids are now emerging on the recreational opioid market, rendering the landscape highly complex and dynamic. While new compounds are entering the supply in rapid and unpredictable manners, some recent patterns have become apparent. Many of these newly emerging opioids are being pirated from early patent literature and/or research papers, synthesized and sold online through various channels. Burdened by the identification of every newly emerging drug, many toxicology labs struggle to keep up. Moreover, by the time a "new" drug is controlled, illicit drug markets will have already adapted and diversified to avoid the restricted product(s). Hence, the typical life-cycle of an NPS opioid is generally short, with only a few drugs escalating to significant numbers of detections. In this work, we review the key events in the emergence, rise, and subsequent downfall of two non-fentanyl opioids – isotonitazene and brorphine – that sequentially dominated the NPS opioid market in 2019 and 2020.

Objectives: Using isotonitazene and brorphine as examples, we illustrate the characteristic life-cycle of synthetic opioids in the 'post-fentanyl-analogue' era. We review key events from their earliest synthesis as described in scientific literature, to their subsequent rise and fall on recreational markets.

Methods: A literature review was conducted to collect the key events related to the life-cycles of isotonitazene and brorphine.

Results: Isotonitazene was initially synthesized in the 1950s-60s, and was first identified on the recreational market by our group in August 2019. Further investigation brought to light that isotonitazene was circulating since at least March 2019. Alarmed by the many adverse events involving isotonitazene (over 250 fatalities have been identified in the US), the drug was eventually internationally scheduled in April 2021. Cases with isotonitazene reached a peak around the first half of 2020, after which positivity started to decline. Around that time, brorphine started to surface as isotonitazene's successor. Initially described in scientific literature in 2018, the first brorphine case was confirmed by our group in February 2020. Following a rapid rise in positivity, the DEA announced restrictions on brorphine in December 2020. By then, brorphine had started to wane in positivity. While the typical life-cycle of NPS opioids is relatively short (6-12 months), isotonitazene and brorphine both remained in circulation for over a year, each contributing to hundreds of deaths and adverse events. *In vitro* μ-opioid receptor activity determination performed at our lab confirmed that both opioids are highly potent and efficacious.

Conclusion/Discussion: The rise and fall of isotonitazene and brorphine illustrates the persistent dynamic nature of the recreational opioid market. A virtually endless supply of drugs with opioid effects has been described in literature, and minor structural modifications give rise to many closely-related, yet unregulated analogues. Hence, once scheduling impedes one opioid's availability, the emergence of various alternatives designed to evade previous restrictions appears inevitable – a phenomenon observed repeatedly in this work. This stresses the importance of intensive monitoring and a timely, accurate and multidisciplinary response of the international community.

POSTER

P-008: Synthesis, chemical characterization, and μ-opioid receptor activity assessment of the emerging group of 'nitazene' 2-benzylbenzimidazole synthetic opioids

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Background/Introduction: Several 2-benzylbenzimidazole opioids (also referred to as 'nitazenes') recently emerged on the illicit drug market. The most frequently encountered member, isotonitazene, has been identified in multiple fatalities since its appearance in 2019. Although isotonitazene was recently put under international control, many other analogues remain unregulated. Apart from a series of research articles exploring their analgesic potential in the 1950s-1960s, little is known about the harm potential of these increasingly encountered non-fentanyl opioids. In this study, 10 nitazenes and 4 metabolites were synthesized and extensively characterized analytically and pharmacologically. By doing so, this study lays out an analytical framework for the detection of these emerging opioids as well as providing important new insights into their *in vitro* μ-opioid receptor (MOR) activation potential.

Objectives: This study focuses on the MOR activation potential of a set of emerging 2-benzylbenzimidazoles, including three metabolites of isotonitazene and one metabolite of the highly potent etonitazene. In addition, advanced chemical characterization provides the first framework for improved screening of a broad panel of nitazenes.

Methods: Fourteen 2-benzylbenzimidazoles were synthesized and analytically characterized via nuclear magnetic resonance spectroscopy (^{1}H-NMR), high-performance liquid chromatography coupled to diode-array detection (HPLC-DAD), gas chromatography mass spectrometry (GC-MS) and liquid chromatography coupled to time-of-flight MS (LC-QTOF-MS). The potency and efficacy of the nitazenes was determined via two cell-based MOR activation assays (NanoBiT®): activation of MOR, fused to one part of a split nanoluciferase enzyme, leads to recruitment of either β-arrestin 2 or mini-$G_{\alpha i}$, fused to the other part. This restores the nanoluciferase activity, yielding a bioluminescent signal upon addition of the substrate furimazine.

Results: Based on absorption spectra and retention times, HPLC-DAD allowed differentiation between most analogues. LC-QTOF-MS identified a fragment with *m/z* 100.11 (100.1093-100.1140) for 12/14 compounds, which could serve as a basis for MS-based nitazene screening. MOR activity determination confirmed that nitazenes are generally highly active, with potencies and efficacies of several analogues exceeding that of fentanyl. Our data further show that a number of variations to the general 2-benzylbenzimidazole core drastically impact MOR activity, enabling the assessment of structure-activity relationships. Interestingly, our *in vitro* data correspond remarkably well with the rank order of antinociceptive potencies obtained in early studies in mice.

Conclusion/Discussion: As the presence of non-fentanyl opioids on the illicit drug market continues to rise, it will become increasingly important to rapidly identify and characterize new compounds as they emerge. Nitazenes are among the newest to appear and, given their high potential to activate MOR, their use may pose an imminent threat to any user. Particularly relevant is the unexpected very high potency of the *N*-desethyl-isotonitazene metabolite, rivalling the potency of etonitazene and exceeding that of isotonitazene itself. Supported by its identification in fatalities, this likely has *in vivo* consequences. The extensive chemical and pharmacological characterization performed in this study may contribute to increased awareness and detection of this potentially highly dangerous class of emerging synthetic opioids.

P-009: Method Development for Analysis of Cannabis Exposure and Oxidative Stress Biomarkers in Exhaled Breath Condensate and Oral Fluid

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Background/Introduction: According to the 2019 National Survey on Drug Use and Health conducted by the U.S. Department of Health and Human Services (HHS), marijuana is the most popular illicit drug in the U.S. There is a need to investigate biomarkers that differentiate between licit and illicit cannabis consumption, as many states continue legalization for both medicinal and recreational use. Forensic toxicologists seek to identify biomarkers of cannabis exposure that can reliably indicate recent and/or long-term cannabis use, be sampled with relative ease, and correlate with impairment - unlike Δ^9-tetrahydrocannabinol (Δ^9-THC). Clinicians are also interested in determining whether inhalation of cannabis can exacerbate or provide protection from oxidative respiratory stress. Exhaled breath condensate (EBC) and oral fluid (OF) are alternative, non-invasive sample matrices that hold promise for identification of cannabis exposure biomarkers. EBC is an aqueous specimen consisting of condensed water vapor, volatiles, and non-volatiles. OF is currently being explored as a matrix for cannabis exposure analysis, and there are no reports on the use of EBC for this purpose, although other drugs have been detected. Potential advantages of EBC as a sample matrix include its ease of collection and the presence of a wide variety of volatile and non-volatile analytes, including metabolites and markers of respiratory impairment.

Objectives: This work seeks to develop reliable analytical methods that quantify major and minor cannabinoids and their metabolites as potential cannabis exposure biomarkers in EBC and OF. In addition, methods were developed to profile selected respiratory oxidative stress biomarkers in EBC. These methods will be employed in future studies to develop reliable exposure and respiratory stress biomarkers in a cohort of cannabis smokers and non-smokers.

Methods: A total of 20 cannabinoids and metabolites were targeted for analysis based on detections reported in human specimen matrices, inclusion of major and minor cannabinoids and acid precursors, compounds identified in recreational and/or medicinal cannabis, and availability of standards. Seven internal standards were also included. Five oxidative stress biomarkers selected for analysis included 8-isoprostane, 8-oxo-7,8-dihydroguanine, 8-oxo-7,8-dihydro-2'-deoxyguanosine, 4-(methylnitrosoamino)-1-(3-pyridyl)-1-butanone, and 4-(methylnitrosoamino)-1-(3-pyridyl)-1-butanol. An Agilent 1290 Infinity UHPLC coupled to an Agilent 6470 Triple Quadrupole LC-MS/MS with Jet Stream ESI technology was utilized for this work.

Results: All chromatographic separations in the final method were optimized using an Agilent Zorbax 120 EC-C18 column (3.0 x 100 mm, 1.8 μm). Mobile phase A was 5 mM ammonium formate with 0.1% formic acid in water and mobile phase B was 0.1% formic acid in75% acetonitrile: 25% methanol. Column temperature was 40°C and flow rate was 0.2 mL/min. Elution step gradient was 40% B for 5 min, then increased to 70% B at 5 min, then to 75% B at 11 min, then to 80% B at 13 min, then to 98% B at 18 min until 24 min. The ESI source was operated in positive mode. The established dynamic Multiple Reaction Monitoring (dMRM) method for the cannabinoids involves a 26-minute run time and is able to separate all target compounds analyzed in OF and EBC. This method allows LC separation of Δ^8-THC, Δ^9-THC, cannabidiol, cannabichromene, and cannabicyclol, which have same MRM transitions. The dMRM method was effective in analyzing five representative oxidative stress biomarkers for future EBC studies.

Conclusion/Discussion: We developed an LC-MS/MS dMRM method that identifies and distinguishes 20 cannabinoids and their metabolites, and five oxidative stress markers as part of ongoing research into differentiating recent or long-term cannabis consumption, licit or illicit use, and biomarkers that correlate with impairment. The developed analytical methods will facilitate cannabis exposure and respiratory stress biomarker studies in a human cohort of medical and recreational cannabis smokers with different cannabis use profiles.

POSTER

P-010: An Evaluation of Weak Anion Exchange Solid Phase Extraction Cartridges for the Quantitation of PFAS Compounds in Human Biological Matrices

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Introduction: Per- and polyfluoroalkyl substances (PFAS) encompass a large group of manufactured compounds that have been used in various production processes such as food packaging, commercial products, workplaces, homes, water supplies, and in food. PFAS are persistent, resistant to degradation, and can bioaccumulate. The CDC's 2015-16 health survey found average blood levels of 4.72 ng/ml for PFOS and 1.56 ng/ml for PFOA, although an exposure limit that predicts adverse health effects has yet to be determined.

Objectives: Sample preparation and analytical methods are necessary to detect and quantitate these compounds in human biological matrices and help us fully understand how they relate to a variety of health outcomes such as pre- and postnatal health, immunity, cancer, and hormone disruption.

Materials and Methods: All samples and quality controls were prepared by spiking certified reference material into pooled human serum. A laminar flow ultra-high pressure QSight®220 LC-MS/MS was equipped with a Selectra C18 column. Extraction was accomplished using a Weak Anion Exchange (WAX) solid phase extraction (SPE) column (UCT, ECWAX053) by first conditioning the columns with 1 mL of methanol followed by 1 mL 100 mM pH 7 phosphate buffer. Samples were loaded onto the column at a rate of 1-2 mL/min. The SPE cartridges were washed with 1 mL of 100 mM pH 7 phosphate buffer and 1 mL of DI water (Millipore Milli-Q Ultrapure Type 1 water system), then dried under full vacuum for 5 minutes. Elution was carried out with 2.5mL methanol. All samples were reconstituted in 100 μL of 10 mM ammonium acetate solution in DI water. Parameters assessed followed ASB 036 standard for method validation, including matrix recovery, limit of detection (LOD), limit of quantitation (LOQ), and calibration model.

Results: The results of the study were gathered from the following thirteen analytes: PFBA, PFBS, PFHxA, PFHxA, PFHpA, PFHxS, PFOA, PFOS, PFNA, PFDA, PFUnA, PFDoA, PFTrDA. Depending on the analyte, a lower limit of quantitation was established at 0.47 - 2.36 ng/mL and an upper limit of quantitation at 49.5 ng/mL. Based on the established linear calibration model an LOD in the range of 0.107-0.121 ng/mL and an LOQ in the range of 0.47-2.36 ng/mL were achieved. Most PFAS analytes showed an acceptable bias of ±20% except for PFBS (23%), PFHpA (56%), and PFHxS (144%). Concurrent precision calculation (%CV) showed a maximum acceptable range of ±20% for all analytes except for PFHxA (30%) and PFHpA (46%). The average recovery for SPE ranges from 30-80% with recovery of ~30-55% for PFBA, PFHpA, PFHxS, PFOA, PFDA, PFUnA, and 55-80% for PFHxA, PFOS, PFNA.

Conclusions: Utilizing the UCT WAX SPE column, we were able to demonstrate good recovery for the majority of the PFAS compounds. Further, the extraction technique was efficient for high throughput analysis with the extraction time comparable to other traditional SPE methods. The total analytical run time using the UCT Selectra C18 column, 11 minutes, allowed for adequate re-equilibration and system washes to prevent carryover and contamination of these persistent pollutants with excellent chromatography. Accurate quantitation of PFAS compounds in biological matrices will allow for better understanding of prevalence, bioaccumulation in biological matrices and how these concentrations relate to various health outcomes.

POSTER

P-011: Considerations in Establishing ISTD Acceptance Criteria for Volatile Quantitation Using Headspace Gas Chromatography/ Flame Ionization Detection

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Background/Introduction: The newly revised American Board of Forensic Toxicology accreditation standards require monitoring of internal standard (ISTD) recovery for all chromatographic assays, including volatile analysis, and requires documented action when the recovery is below 50%. Based on the internal standard dispensing pipettes' excellent precision and past casework performance, our laboratory attempted to establish a more conservative ISTD response criterion of 80-120% of the average calibrator ISTD response. However, while testing this new range, samples intentionally prepared with different volumes of ISTD (e.g., approximately 30% of normal and 200% of normal) produced responses within the acceptable range. We initiated an investigation to understand why ISTD responses were not performing as expected.

Objectives: To evaluate factors contributing to ISTD responses and successfully establish a narrower response range for the ISTD acceptance.

Methods: Headspace gas chromatography flame ionization detection (HS/GC FID) was used to quantify methanol, ethanol, isopropanol and acetone in blood. The routine procedure employed a Hamilton 600 series dual syringe dilutor with 250 and 2500 µL syringes to pipette 100 µL of sample and 700 µL of 0.02% n-propanol ISTD into a 20 mL headspace vial. The headspace autosampler conditions included an oven and loop temperature of 70 °C and transfer line temperature of 100 °C, 7-minute vial equilibration and an injection duration of 0.50 minutes. The GC inlet had a temperature of 180 °C with a split ratio of 10:1. The column oven is held at 40 °C. Experimental conditions included preparing known blood controls with varying amounts of ISTD (500 µL, 700 µL, 900 µL, and 1400 µL) in 10 or 20 mL headspace vials, using different syringe sizes (1000 µL and 100 µL) and using ISTD with and without ammonium sulfate. Contemporaneously analyzed aqueous calibrators were used to generate average ISTD response ranges of 80-120%, 90-110%, and 95-105%. ISTD area response in the blood controls were compared to these ranges.

Results: Across all experimental conditions, decreasing or increasing the volume of ISTD did not proportionally decrease or increase ISTD response. The 80-120% and 90-110% ISTD ranges were too broad to identify changes in the ISTD volume; therefore, the 95-105% average ISTD response was chosen as the most appropriate acceptance range. No single change in autosampler vial size, syringe size, or ISTD salt modifier produced results that would meet the narrower range of acceptance criteria. The combination of 10 mL autosampler vials and ISTD with ammonium sulfate was the only experimental condition in which insufficient or excess ISTD yielded unacceptable ISTD responses.

Conclusion/Discussion: Internal standard responses should reflect errors in internal standard delivery, extraction, instrumental analysis and/or matrix effect. This work sought to identify which method conditions and internal standard acceptance criterion would indicate a problem with a particular sample. Preliminary work indicates that a smaller autosampler vial, salt modifier, and a narrower internal standard acceptance criterion may be necessary. A smaller headspace volume may allow the sample to achieve equilibrium faster and the salt modifier alters the partition coefficient for the volatile compounds. Additional testing will be necessary to confirm that equilibrium is being reached and whether the new analytical conditions are also appropriate for non-blood matrices.

POSTER

P-012: Fast Screening Method for Drug Facilitated Sexual Assault Drugs by Direct Mass Spectrometric Analysis of Dried Urine Spots

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Background/Introduction: Screening methods are routinely used in toxicology labs to quickly determine whether a sample is presumptive positive for drugs. Current screening methods commonly employed (immunoassays) may lack specificity and scope in certain forensic cases, such as drug-facilitated sexual assault (DFSA) cases. In DFSA cases, the preferred biological sample for the analysis of drugs is urine, as it offers a longer window of detection (several days) compared to blood (several hours). In recent years, analytical techniques using dried matrix spots have been increasingly developed and used, due to the low biohazard risk and the easy storage (a card at room temperature) of the dried samples.

Objectives: The aim of this research was to assess the direct analysis of dried urine spots (DUS) by mass spectrometry (MS) as a fast, specific and sensitive screening method for DFSA drugs. The drugs investigated in this study included gamma-hydroxybutyrate (GHB), pregabalin, gabapentin, valproic acid, carisoprodol, meprobamate and topiramate.

Methods: Whatman® 903 protein saver cards were fortified with sample and analyzed via a Plate Express coupled to a single quadrupole compact mass spectrometer expression® CMS from Advion (Ithaca, NY). Drugs were individually optimized for ion mass-to-charge ratios (m/z), ion source parameters, and injection parameters (mobile phase and extraction head parameters) by the plate reader extraction of dried spots of methanolic standards at 10 µg/mL. Cut-offs were investigated dispensing 20 µL of methanolic solution or urine sample (10 different donors) fortified at a wide range of concentrations (0.01-10 µg/mL) into the center of the spot on the card and allowed to dry (10 min for methanolic samples, overnight for urine samples) at room temperature before analysis. After drying, the spot was cut from the card and placed on a clean microscope slide for analysis to avoid cross-contamination between spots. Endogenous interferences were evaluated analyzing 10 negative urine samples. Exogenous interferences among the target analytes were investigated at 1 µg/mL.

Results: The optimized m/z were 143 for valproic acid; 103.1 for GHB; 261.3 for carisoprodol; 338.1 for topiramate; 160.3 for pregabalin; 154.1, 137.1 and 172.2 for gabapentin; 219.2 and 158.1 for meprobamate. Topiramate, GHB and valproic acid were analyzed in negative ion mode, while carisoprodol, pregabalin, gabapentin and meprobamate were analyzed in positive ion mode. The mobile phases that yielded the best signal-to-noise ratio were 0.1% formic acid in acetonitrile (positive mode), and methanol (negative mode). The optimized extraction time for the Plate Express was 60 s. No endogenous nor exogenous interferences were observed for any of the target analytes. The cut-offs of the methanolic solution spots were 0.01 µg/mL for gabapentin, 0.25 µg/mL for topiramate, carisoprodol and pregabalin, 0.5 µg/mL for valproic acid and 1 µg/mL for GHB and meprobamate. The cut-offs in DUS were 0.025 µg/mL for gabapentin, 0.25 µg/mL for topiramate and pregabalin, 0.5 µg/mL for valproic acid and carisoprodol and 1 µg/mL for GHB and meprobamate.

Conclusion/Discussion: Direct analysis of DUS by MS proved to be a promising screening technique for DFSA drugs in urine. This technique saves time, allowing the simultaneous analysis of different drugs with sensitivity and specificity. The employment of DUS facilitates shipping and reduces storage constraints (cards are stored at room temperature) and biohazard risks in toxicology labs.

POSTER

P-013: Fast Analysis of 28 Benzodiazepines and Metabolites in Hydrolyzed and Non-Hydrolyzed Urine by LC-MSMS

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Background/Introduction: Benzodiazepines, a drug group of tranquilizers with sedative, hypnotic properties, are commonly encountered in different types of forensic cases, such as overdoses and in victims of drug facilitated sexual assault (DFSA). Fast and easy-to-use multi-analyte procedures covering a wide analytical range and achieving the required sensitivity for challenging cases, such as DFSA, are necessary.

Objectives: The objectives of this work were: 1) to develop fast and simple analytical methods for the simultaneous determination of 28 benzodiazepines and metabolites in hydrolyzed and non-hydrolyzed urine, and 2) to compare the hydrolysis efficacy and performance of 2 rapid enzymes, B-One™ recombinant b-glucuronidase and BG-Turbo® glycerol free high efficiency recombinant b-glucuronidase (Kura Biotech, Puerto Varas, Chile). The target analytes were: 3-hydroxyflubromazepam, 3-hydroxyphenazepam, 7-aminoclonazepam, 7-aminoflunitrazepam, alpha-hydroxy-alprazolam, alpha-hydroxy-midazolam, alpha-hydroxy-triazolam, alprazolam, bromazepam, clobazam, clonazepam, delorazepam, desalkylflurazepam, diazepam, etizolam, flubromazepam, flunitrazepam, flurazepam, lorazepam, lorazepam-glucuronide, midazolam, nordiazepam, oxazepam, oxazepam-glucuronide, phenazepam, temazepam, temazepam-glucuronide, and triazolam.

Methods: For the non-hydrolyzed urine samples, 50 uL of urine were transferred into the shell vial of a nanoFilter Vial™ (PES 0.2 um) from Thomson Instrument Company (Oceanside, CA), fortified with 25 uL of IStd mixture (9 deuterated analogs) at 100 ng/mL prepared in water, and 150 uL of water were added. The plunger with filter was slightly inserted into the shell vial, vortexed and then inserted all the way. The filtered sample was directly injected into the LC-MSMS. No evaporation step was required. For the hydrolysis with B-One b-glucuronidase, we mixed into the shell of the nanoFilter Vial 50 uL of urine, 25 uL IStd mixture, 50 uL of water and 100 uL of the enzyme. After waiting 5 min at room temperature, the samples were filtered as described and injected into the LC-MSMS. For the hydrolysis with BG-Turbo b-glucuronidase, we mixed into the shell vial 50 uL of urine, 25 uL IStd mixture in water, 20 uL buffer, 10 uL of the enzyme and 120 uL of water. No waiting time was required before filtration. The methods were evaluated for linearity, limits of detection (LOD) and quantification (LOQ), extraction efficiency, matrix effect and interferences. The methods were applied to 26 authentic urine samples from benzodiazepine users.

Results: All the methods, without hydrolysis and with hydrolysis with both enzymes, were linear between 5 and 100 ng/mL, with LODs between 1 and 5 ng/mL, depending on the analyte. No loss was observed within +/- 20% for any of the 28 analytes due to filtration with PES filters. However, differences were observed among the matrix effects (n=10, at 10 and 100 ng/mL). The non-hydrolyzed samples and the BGTurbo samples showed ion enhancement (up to 51.2%) for 4 and 3 analytes, respectively. The B-One samples showed ion enhancement (up to 56.5%) for 2 analytes, and suppression (up to -54.4%) for 13 compounds. No exogenous nor endogenous interferences were observed (n=10). In fortified samples with lorazepam-glucuronide, oxazepam-glucuronide and temazepam-glucuronide at 10 and 100 ng/mL, both enzymes performed a complete hydrolysis. In the authentic cases, the hydrolysis with both enzymes also allowed the detection of the glucuronides (non-hydrolyzed sample concentration vs. hydrolyzed sample concentration) of midazolam (0 vs. 62.5-102 ng/mL), hydroxy-midazolam (0-12.9 vs. >100 ng/mL), hydroxy-alprazolam (0-4.7 vs. >100 ng/mL) and nordiazepam (0-99.6 vs. >100 ng/mL).

Conclusion/Discussion: We described a fast and easy procedure for the analysis of 28 benzodiazepines in urine. The sample preparation consisted in sample dilution and filtration within the injection vial, and the enzymatic hydrolysis was performed in the vial as well at room temperature in less than 5 min. Both enzymes showed excellent efficacy, although the B-One enzyme produced higher matrix effects in urine than non-hydrolyzed samples and the BGTurbo enzyme.

POSTER

P-014: Untargeted Poisonings of Southern African Wildlife

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Background/Introduction: Over history, pesticides have been used widely on the African continent against wildlife. They are used to protect crops and farmland against insects, pest, and wild animals that attack livestock. Poachers are also known to use pesticides while hunting big game. The use of pesticides as a means to kill animals however causes inadvertent poisonings of other species such as scavengers who feed from the dead carcass, or if an unintended animal ingests the poisoned bait (e.g., fruit, crops) that are left behind.

Objectives: The objectives of this project consisted of developing a method to extract pesticides from organic material (e.g. stomach contents, tissue, fruit) in suspected animal poisonings into a medium suitable to be shipped from Africa to the United States (US), using common household chemicals that could be sourced locally, and developing an analytical method to analyze the samples using liquid chromatography tandem mass spectrometry (LC-MS/MS).

Methods: In-field extraction was performed on different biological and non-biological matrices from suspected animal poisonings and extracted into a paper towel using acetone. The samples were allowed to dry and shipped to the US. Upon receipt, a 2 by 2-inch piece of the paper towel was cut and passively extracted with 5 mL of ethyl acetate. Samples were dried down and reconstituted in mobile phase then centrifuged and transferred to autosampler vials to remove any debris. Analysis was conducted on a Waters™ Acquity-UPLC, Waters Xevo. The analytical column was an Acquity UPLC® BEH-C18 (1.7 µm, 2.1x100 mm) at a temperature of 45°C, with ammonium formate (pH=4) in water (MPA) and 0.1% formic acid in methanol (MPB) was used. This method allowed for the chromatographic resolution and qualitative identification of monocrotophos, 3-hydroxycarbofuran, carbofuran, aldicarb, aldicarb sulfoxide, aldicarb sulfone, carbaryl and methomyl. The method was validated using a fit-for-purpose validation adapted from the American Standards Board (ASB) validation standard including: limit of detection (LOD), recovery, carryover, interferences, and stability at 23°C, 33.9°C and -80°C. A total of 105 samples were collected and analyzed during a 4-year period (2016-2020) from several nature reserves in Botswana and Zimbabwe.

Results: All validation parameters were met with acceptable criteria and the compounds were stable at all temperatures for 14 days. All compounds have a recovery greater than 50%. From the 105 samples received, a total of 24 samples tested positive for one or more pesticides from 12 different incidents: elephant(n=3), vulture (n=2), hippopotamus (n=2), warthog (n=1), buffalo (n=1), knob-billed geese (n=1), bateleur eagle (n=1), and kudu (n=1). Fruit was also collected in two cases. On one incident, an elephant was suspected of being poisoned and to have caused secondary poisoning of 104 vultures (2 vulture sample tested, and 1 elephant sample tested from the same case). The elephant tested positive for aldicarb, aldicarb sulfoxide, carbofuran, 3-hydroxycarbofuran, trace methomyl, trace carbaryl, whereas the vulture samples tested positive for all but Methomyl. In another case, a Hippopotamus sample (n=1) was analyzed. The incident involved 12 dead hippos belonging to the same pod during a 5-day span. Suspected poisoning source was from a small irrigation system in the area. The test results were positive for aldicarb, trace aldicarb sulfoxide and carbofuran.

Conclusion/Discussion: Pesticides have been used for decades, but some animals end up as indirect casualties of these poisonings. The case histories show how secondhand poisoning and habitat contamination are a major issue that kills animals indiscriminately and can create a chain reaction that could kill hundreds more unknowingly. Being able to detect and report these cases to the African wildlife agencies can help to prosecute the perpetrators.

POSTER

P-015: Survey of US Residents and Their Usage of Electronic Cigarettes with Drugs Other Than Nicotine

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Background/Introduction: Electronic cigarettes (e-cigs) were originally designed as an alternative to smoking combustible cigarettes. Early e-cig liquids (e-liquids) were typically composed of some mixture of propylene glycol (PG) and vegetable glycerin (VG) as carriers, chemicals to produce a desired flavor profile, and nicotine. E-liquids commercially available have claimed to contain dietary supplements such as vitamins, melatonin, and herbal remedies. Chemical analyses of commercial products have identified mitragynine, nuciferine, and apomorphine, and also demonstrated adulterations with MDMB, 5F-ADB, and dextromethorphan. Surveillance of internet drug forums have demonstrated interest and associated "expertise" in adding illicit and controlled substances to e-liquids. E-cigs are generally accepted, and vaping does not produce a distinct smell to indicate the presence of drugs other than nicotine (DOTN), facilitating a discrete administration of psychoactive drugs. Describing and understanding evolving use, trends, and prevalence of e-cigs as an alternative tool for consuming DOTNs is critical to inform stakeholders in public health and public safety of this potential use of e-cigs and its implications.

Objectives: The purpose of this study is to elucidate which e-cig devices are being used to vape what substances, as well as frequency, context of use, and general population demographics. A survey of current e-cig users in the United States over the age of 18 who use e-cigs to "vape" DOTNs has been disseminated in online forums.

Methods: A survey (IRB HM20012698) was designed to collect demographics, device and DOTN use, frequency and context of use, and whether users typically purchase e-liquids with the DOTN or make homemade formulations. No incentives are offered for completion to protect anonymity. The survey was deployed by posting on BlueLight and sub-Reddit forums specific to recreational drug use, Craigslist, and the social media sites Facebook and Instagram.

Results: The original posting on BlueLight was unproductive. Postings on various sub-Reddits produced few participants, but generally was not well received and was not found to be an effective recruitment tool. Craigslist postings were also ineffective, as posts were taken down typically within 30 minutes for unknown reasons. In addition to ineffective deployment strategies, surveys are not always taken to completion. Of the 157 surveys started, 35 were taken to completion, 47 were partially completed, and 75 were not eligible due to age or location. Of the 35 completed surveys, eligible participants were 18-70 years of age and predominantly identified as Heterosexual (21) White American (31) Male (25). Data indicates that DOTNs are currently being used in e-cig devices. DOTN classes indicated as "ever used" with e-cigs include: cannabidiol (N=21), delta9-tetrahydrocannabinol (N=34), "other cannabinoids" (N=7)), amphetamines (N=1), herbal products (N=4), kratom (N=2), caffeine (N=2), vitamins (N=2), and dimethyltryptamine (N=3). Context of use has been indicated as: alone, with friends, at social events, only when drinking alcohol, while driving, at home, at work, at school, indoors, and outdoors.

Conclusion/Discussion: The deployment and completion of the survey was challenging in user forums, especially without an incentive for completion. To increase visibility, postings needed to incorporate relevant hashtags. Users indicated regular-to-daily DOTN use in e-cigs. Reported use while driving, at work, or at school highlighted the value of discreteness and social acceptability of e-cigarettes. Additionally, the findings support the continual need to educate public health and safety officials of the use DOTNs in e-cigarettes.

POSTER

P-016: Ethanol stability from five years of a blind quality control program in blood alcohol analysis

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Background/Introduction: Existing literature on ethanol stability in vitro describes variables such as storage temperature (e.g. refrigerated, ambient, or elevated) and presence of preservatives. Despite these differences, studies concluded that over time ethanol decreases in antemortem blood concentration, and the rate of decrease is dependent largely on storage conditions, sample volume, and prior opening of the sample containers.

Blind quality control (BQC) samples offer the advantage of monitoring the stability of ethanol in a format that is blinded to the analyst and collected/processed identically to authentic casework, including intermittent periods at room temperature wherein case samples are routinely transferred to/from the submitting agency and accessioned.

Objectives: To determine whether case samples stored refrigerated but untested for long periods as in a backlog still produced results that aligned closely with ethanol concentrations at the time of blood draw (e.g., within ±5% of the target concentration).

Methods: The design and operation of Houston Forensic Science Center's (HFSC) BQC program has been previously described. Briefly, grey top tubes containing approximately 7 mL human whole blood fortified with ethanol to yield ten target concentrations were manufactured between June 2015 and August 2019 (0, 0.08, 0.10, 0.13, 0.15, 0.16, 0.18, 0.20, 0.24, and 0.25 g/100 mL). HFSC's Quality Division submitted the BQC samples into the casework workflow in evidence kits identical to those of authentic case samples. BQC samples were analyzed by dual-column headspace gas chromatography with flame ionization detection between October 2015 and February 2021. Values were reported from averaging the results of two samplings in one analytical run. Results were grouped by length of time from date of manufacture to analysis in three-month intervals (0-90, 91-180, 181-270, 271-360, 361-450, 451-540, 541-630, and >630 days). Results reported were compared to the manufacturer's target values to calculate percent difference.

Results: For 746 BQC cases, the average (min-max) time that passed between manufacture and analysis was 342 (42-758) days. All 203 cases with target ethanol concentrations of 0 g/dL were correctly reported as none detected regardless of the age of the item (42 to 758 days). Generally, average %difference increased over time, and higher target concentrations exhibited less loss than lower ones.

Manufacture to analysis (days):		0-90	91-180	181-270	271-360	361-450	451-540	541-630	>630
Target (g/dL)	**N**	**Average percent difference (%)**							
0.00	203	N/A							
0.08	142	-2.8	-3.7	-2.4	-5.0	-5.9	-5.8	-	-
0.10	58	-	-1.3	-1.5	-4.1	-3.4	-4.3	-4.5	-
0.13	53	-0.5	0.0	-1.2	-2.5	-4.3	-4.8	-	-
0.15	45	-	-3.2	-4.0	-4.0	-4.1	-4.9	1.4	-6.5
0.16	47	-0.3	-0.8	-1.3	-2.4	-2.3	-1.4	-	-
0.18	58	-	-0.6	-0.6	-1.1	-1.3	-2.1	-1.6	-
0.20	43	-	-1.9	-2.1	-2.8	-2.4	-3.3	-0.1	-3.6
0.24	55	2.4	2.6	1.6	1.2	1.1	1.0	-	-
0.25	42	-	0.5	0.7	-0.7	-0.7	-0.8	0.3	-1.2

Conclusion/Discussion: Results obtained were generally in agreement with existing literature on stability of ethanol in vitro. The greatest %difference observed, -6.5%, was for the 0.15 g/dL target concentration and was still within the uncertainty of measurement for our analytical method (9.6% at k=3). In addition to confidence in reporting alcohol results after an extended backlog, the data suggest that samples prepared for a BQC program for alcohol analysis are acceptable for use well beyond one year. Limitations to our study design include the same approximate amount of blood in each sample tube and the BQCs were prepared in a laboratory setting, and so variables introduced from blood draw were not considered.

POSTER

P-017: Long- and short-term stability of methylphenidate and its metabolites in blood

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Background/Introduction: Methylphenidate (MPH) is a common prescription medication prescribed for attention-deficit hyperactivity disorder (ADHD), but may also be abused recreationally. Though MPH has two chiral centers, d-threo-MPH is responsible for the pharmaceutical effects. MPH is typically prescribed at a dose of 20-30 mg. Maximum concentrations (C_{max}) following 18mg/day Concerta® were 3.7 ± 1.0 ng/mL in plasma. Few studies have analyzed methylphenidate and its metabolites, ritalinic acid (RA) and ethylphenidate (EPH), in blood. Stability studies are crucial in a forensic setting to provide insight on ideal storage conditions and analysis time for forensic samples. This is the first method to our knowledge that analyzes the long-term stability of d,l-MPH, d,l-EPH and RA in blood.

Objectives: The goal of this study was to determine the stability of d,l-MPH, d,l-EPH and RA in blood over a 9 month period in various settings. In addition, we sought to assess MPH degradation to RA over time.

Methods: Blood (preserved with sodium fluoride and potassium oxalate) was fortified with d,l-MPH, d,l-EPH and RA at 15ng/mL and 150ng/mL to represent low quality control (LQC) and high quality control (HQC), respectively. Blood samples were evenly distributed into vacutainer tubes and stored at the following conditions: room temperature (~25°C), refrigerated (4°C), frozen (-20°C) and elevated temperature (35°C). Analytes were quantified using isotopically labeled internal standards (d,l-MPH-d10 and RA-d10) and a previously validated liquid chromatography tandem mass spectrometry method following solid phase extraction. Baseline concentrations were calculated by analysis immediately after preparation (T_0). Samples were analyzed in triplicate after 24h, 48h, 72h, 1wk, 2wk, 3wk, 6wk, 2mo, 3mo, 4mo, and 5mo. In order to assess MPH degradation to RA, blood was fortified at LQC and HQC concentrations with d,l-MPH and then stored under the same temperature conditions and analyzed in duplicate over 8 time points (T_0, 24h, 48h, 72h, 1wk, 2wk, 3wk, 1mo). From the established baseline, stability was assessed as a %difference from this value by dividing the calculated concentration at each time point by the baseline concentration and considered acceptable within ±20%.

Results: At room temperature, RA concentrations increased 53% within 24 hours while d- and l-MPH concentrations dropped 18.1% and 20.6%, respectively, in the same timeframe. Under refrigeration, all analytes were stable for one week (±17.1%). All analytes were considered stable for 5 months at frozen temperatures. At elevated temperatures, RA concentrations increased 244% in the HQC while l-EPH remained stable for 24h (14.4% loss). All other analytes displayed instability within 24 hours. Due to rising RA concentrations, a follow up study was conducted to assess the breakdown of MPH. At room and elevated temperatures, MPH broke down completely to RA within two weeks. At both refrigerated and frozen temperatures, MPH degradation to RA was apparent but MPH was still detectable.

Conclusion/Discussion: The long-term study revealed MPH and EPH instability at all conditions, except when blood was stored frozen. As such, the optimal storage for samples suspected of containing these analytes is recommended at -20°C. From the short-term study, it was concluded that d,l-methylphenidate breaks down in the blood to its metabolite ritalinic acid. This may make data interpretation difficult as quantitative values may be inaccurate if forensic samples are not properly handled and stored. This study shows the importance of stability studies and gives useful information on storage conditions for these analytes in blood.

POSTER

P-018: Effective Separation of Cannabinoid Isomers in Blood

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Background/Introduction: The prevalence of cannabis is typically second only to alcohol in forensic casework such as driving under the influence of drugs (DUID). Delta-9-tetrahydrocannabinol (delta-9 THC), and its metabolite 11-nor-9-carboxy-delta-9-tetrahydrocannabinol (delta-9 carboxy THC), are commonly detected following cannabis use. In cannabis, there exists delta-9 THC, and to a much smaller degree, its isomer delta-8 THC. Because of the relatively low concentration of delta-8 THC, it was previously not necessary to separate the two isomers, or their respective metabolites, in forensic analysis. Furthermore, multi-class methods designed for polypharmacy casework may lack the selectivity to separate these isomers. However, recently, cannabis distributors are deriving mostly pure delta-8 THC from cannabidiol extracted from hemp as a legal alternative to delta-9 THC. Because these products are now available, it can no longer be assumed that a sample positive for THC or carboxy THC using a method that does not separate the isomers only includes insignificant amounts of delta-8 isomers. Because delta-8 THC is less potent than delta-9 THC, it is now necessary to be able to distinguish these isomers and their metabolites from one another in DUID or other casework where impairment or litigation is relevant.

Objectives: The goal was to develop a method that can be undertaken immediately following a positive detection of THC or carboxy THC in a multi-class method not designed for cannabinoid isomer separation, without any additional sample preparation.

Methods: This method is designed to immediately follow, without additional sample preparation, a technique that detects but lacks specificity for THC and carboxy THC. Sample preparation consists of protein precipitation followed by size exclusion Thomson filtration of 150 µL of blood**.** Separation and acquisition are performed in 7.5 minutes using an LC-MS/MS equipped with a Phenomenex Kinetex 2.6 µm 100 Å PS C18 100 x 2.1 mm column. The LC-MS/MS system consists of a Sciex Nexera X2 LC-30 coupled with a Sciex QTRAP 6500+ mass spectrometer utilizing an Ion Drive™ Turbo V electrospray ionization (ESI) source operating in positive multiple reaction mode (MRM).

Results: The final qualitative method for blood analysis was validated according to the ASB/ANSI standards. The method separates delta-8 and delta-9 isomers of THC and carboxy THC, in addition to separating delta-9 THC from other THC isomers cannabichromene (CBC), (6aR,9R)-delta-10 THC, (6aR,9S)-delta-10 THC, 9(R)-delta-6a,10a THC, and 9(S)-delta-6a,10a THC.

Conclusion/Discussion: By creating a subsequent method to add onto an existing multi-class comprehensive technique, the developed and validated method allows for increased selectivity of THC and carboxy THC isomers. The immediate separation of THC isomers following initial cannabis detection is efficient by reducing resource requirements with no additional extractions required.

POSTER

P-019: Overcoming Heterogeneity in Urine Specimens: Avoiding False Negatives caused by Endogenous Inhibitors

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Background/Introduction: Urine drug testing (UDT) is a common practice for monitoring the use of prescribed opioid medications. UDT 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). Screening and confirmatory assays benefit by the application of enzymes that hydrolyze, or deconjugate, glucuronidated analytes. A new generation of beta-glucuronidases can effectively cleave glucuronides in urine at room temperature. However, we have identified additional challenges in urine drug testing across biologically relevant pH extremes and patient urine specimens.

Objective: Screen and characterize the efficiency of new generation room-temperature beta-glucuronidases in a range of clinically-relevant urine and surrogate urine matrices. Identify important factors and key metrics for evaluating enzyme performance with confidence.

Methods: Opioid-positive urine specimens were obtained from a national testing laboratory. Negative controls and clinical specimens were buffered and hydrolyzed with different beta-glucuronidases prior to analysis by liquid chromatography tandem mass spectrometry (LC-MS/MS). Selected specimens were further fortified with known concentrations of glucuronidated drug metabolites to obtain drug-specific hydrolysis efficiencies.

Results: Purified beta-glucuronidases were compared to show enzyme- and matrix-dependent biases on a panel of common drug targets, including opioids (semi-synthetic or non-synthetic), benzodiazepines, illicit substances, and tricyclics across a range of pH. Synthetic urine (Surine) is free of endogenous metabolites that can interfere with drug target hydrolyses, either inhibiting activity and/or inactivating the enzyme. By contrast, certified drug-free urine (DFU) or pooled human urine samples more closely represent clinical samples. Catalytic rates for a single enzyme can vary by two orders of magnitude across the 13 different glucuronidated analytes tested at optimal pH. Thus, monitoring a single analyte as an internal control for enzyme performance can be misleading. We identify key metrics to ensure enzyme performance is properly assessed to achieve high fidelity in urine drug tests.

Conclusion/Discussion: Diluting urine 3-fold or more with buffer yields measurable improvements in achieving target pH and minimizing the impact of endogenous compounds on enzyme performance. A new beta-glucuronidase overcomes many of these challenges and greatly lowers the risk of failed hydrolyses.

POSTER

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P-020: Cutting Down on Confirms with Adjunct Urine Drug Screening using RapidFire-Mass Spectrometry

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Background/Introduction: High throughput urine drug testing programs rely heavily on immunoassays for initial screening of specimens and on GC/MS or LC-MS/MS for confirmatory testing. Due to the higher cost, time investment, and skill required for confirmatory testing methods, military drug testing laboratories often perform adjunct screening on presumptive positive specimens utilizing secondary immunoassay (IA) kits. While this greatly decreases the number of negative samples going to confirmation, certain drug classes (such as amphetamines) have relatively low confirmation rates, making the development of fast but targeted adjunct screening methods desirable.

A recent resurgence of lysergic acid diethylamide (LSD) use among the military population resulted in its reintroduction to the routine drug screening panel for Service Members. With only one military lab certified for LSD confirmation, and the only commercially available IA kit not delivering the desired specificity, the need to develop a targeted LSD adjunct screening method was paramount.

Objectives: Develop fast, targeted adjunct urine drug screening methods for LSD and amphetamines/designer amphetamines (AMPS).

Methods: The AMPS method was designed to target amphetamine, methamphetamine, 3,4-methylene-dioxyamphetamine (MDA), and 3,4-methylenedioxymethamphetamine (MDMA), while the LSD method targeted 2-oxo-3-hydroxy LSD (OHLSD), the primary metabolite of LSD. Urine specimens were plated using a Hamilton MicroLab STAR. For the AMPS method, 1 µL urine was added to 1 mL internal standard diluent (2 ng/mL methamphetamine-D14 in water). For the LSD method, 4 µL urine was added to 1 mL internal standard diluent (10 pg/mL LSD-D3 in 10 mM ammonium carbonate). The plates were mixed on the heater/shaker deck for five minutes, sealed, and transferred to a RapidFire-365 configured with an Agilent 6460 triple quadrupole mass spectrometer for analysis. A C18 RapidFire cartridge was used for sample cleanup for both methods using deionized water (AMPS) or 10 mM ammonium carbonate (LSD). The cartridge was washed [using methanol (AMPS) or 5% acetonitrile (LSD)], samples were eluted and sent to the MS using 0.1% formic acid in 50:50 methanol/isopropanol (AMPS) or 50:50 methanol/isopropanol (LSD). One multiple reaction monitoring (MRM) transition was collected for each analyte and internal standard. Analysis by RF-MS only required 19 seconds per sample for the LSD method and 14.6 seconds per sample for the AMPS method.

Results: Methods were validated for qualitative analysis only; however, validation studies performed included linearity, precision and bias, stability, carryover, matrix effects, and specificity. Sample results were considered invalid if the internal standard response was less than 10% of the calibrator. Approximately 12,000 specimens were analyzed by IA since LSD testing began in December 2020. In that time, 188 samples that screened presumptively positive were subject to an adjunct test using RF-MS. Only ten urine samples screened above the cutoff and were sent for confirmatory testing by LC-MS/MS. An additional two specimens were sent for confirmatory testing due to exhibiting low internal standard response. Of the samples tested by LC-MS/MS, eight confirmed positive for OHLSD and/or LSD and four were shown to be negative. Testing of service member specimens using the AMPS adjunct screen has not yet begun.

Conclusion/Discussion: The RF-MS adjunct screen for LSD reduced the number of negative specimens being sent to confirm by 97.8% and saved a significant amount of time and money compared to confirmatory testing. Samples did not have to be re-poured for analysis, since aliquots tested by IA were simply transferred to the Hamilton for RF-MS plating. No extraction was necessary, and sample preparation required minimal time and resources. Reagent use was further minimized by the short instrument run time. The reduction in time and cost for the RF-MS methods make them an excellent option for laboratories looking to decrease their confirmation workload.

POSTER

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P-021: Enzymatic hydrolysis of >10000 ng/mL of codeine, morphine, ethylmorphine and oxymorphone glucuronides in urine at RT for LC-MS-analysis

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Background/Introduction: Hydrolysis of drug conjugates before analysis facilitates analysis and saves time in data evaluation. Ethylmorphine and codeine glucuronides are however difficult to hydrolyse enzymatically, and typically demand higher temperatures and extended reaction times for acceptable recovery. In drug testing it is also useful to establish the Codeine/Morphine ratio which is typically 10 for Codeine intake, but less if intake was supplemented with Morphine. Thus a correct determination of higher levels of codeine and morphine is useful for interpretation.

Objectives: Find a reasonably quick hydrolysis protocol for drug conjugates of codeine, morphine, ethylmorphine and oxymorphone at >10000 ng/mL each in urine.

Methods: A mixture of morphine-, codeine-, ethylmorphine-, and oxymorphone-glucuronide was spiked into 8 native blank urine samples, giving 20000 ng/mL of each drug conjugate, corresponding to approximately 13000 ng/mL free drug each. Then, 50 µL urine was mixed with 100 µL B-One glucuronidase (Kura Biotech Inc) in a 96-well plate, and left to stand for 30 minutes in RT. Internal standard was added after hydrolysis, in order to keep methanol concentration <10 % in the reaction mixture. Hydrolysis was quenched by adding 275 µL methanol and 100 µL reaction mixture was mixed with 400 µL water before analysis. Samples were analysed using LC-MS/MS. The protocol was repeated on three different days with different native urine samples.

Results: Recovery of free drugs in percent of expected amounts were calculated over the full set of 24 individual native urine samples, as shown below, with corresponding CV's.

Average	Morphine	Codeine	Ethylmorphine	Oxymorphone
Recovery	98%	93%	93%	97%
CV	1.9%	8.8%	8.6%	2.0%

The higher CV's observed for Codeine and Ethylmorphine indicate that individual urine composition has a larger impact on recoveries. However, out of the 24 samples, only one showed <80 % recovery of Ethylmorphine and Codeine.

Conclusion/Discussion: A quick and efficient method for hydrolysis of high levels of Morphine, Codeine, Ethylmorphine and Oxymorphone drug conjugates has been demonstrated. Using B-One glucuronidase (Kura Biotech Inc.) hydrolysis for 30 minutes in room temperature of >10000 ng/mL of each drug resulted in good to excellent recoveries.

POSTER

P-022: Evaluating the Efficacy of Three Beta-Glucuronidase Enzymes for the Detection of Opioids for Forensic Toxicology Urine Testing in Drug Facilitated Crime Investigations

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Introduction: Drug-facilitated sexual assaults (DFSA) are a serious public health and safety concern. Liquid chromatography –tandem mass spectrometry (LC-MS/MS) is an analytical technique that has been previously shown to be effective for detecting and quantifying drugs in human biological samples in DFSA cases. Urine is the preferred sample of choice in these cases, as the detection window for certain analyte metabolites can be detected up to 4 days after the alleged incident. Opioids are of particular concern due to the central nervous system depressant effects and are often excreted in the urine as glucuronidated metabolites. By incorporating an enzymatic glucuronide hydrolysis step in sample preparation, the parent drug of these compounds can be targeted.

Objectives: To evaluate the efficacy of three enzymes for the recovery of parent drug using enzymatic hydrolysis in sample preparation.

Methods: Enzymatic recovery was evaluated for three enzymes: B-One™ shelf-stable β-Glucuronidase for high-throughput analysis from Finden by Kura (Puerto Varas, Los Lagos, Chile), BGTurbo® Glycerol Free High Efficiency Recombinant β-Glucuronidase from Finden by Kura, and Fast β-Glucuronidase, Recombinant from Sigma (St. Louis, MO, USA). See Tables 1-4 for hydrolysis mix and incubation conditions. Sample extraction was performed using supported liquid extraction. Four opioid metabolites were utilized in this research: codeine-6-β-D-glucuronide, dihydrocodeine-6-β-D-glucuronide, hydromorphone-3-β-D-glucuronide, and morphine-3-β-D-glucuronide (Cerilliant, RoundRock, TX, USA). Following sample preparation including hydrolysis and supported liquid extraction (Biotage, Charlotte, NC, USA) , urine case samples were analyzed utilizing the QSight® 220 CR laminar flow tandem mass spectrometer with electrospray ionization, operated in positive ion mode (PerkinElmer, Waltham, MA, USA). Chromatographic separation was achieved using a 50 x 4.6 mm pore size 100 Å, 2.6 μm core-shell Kinetex® phenyl-hexyl HPLC column from Phemomenex® (Torrance, CA, USA). The column was kept at 40°C for the duration of the run. The aqueous mobile phase A was 0.1% formic acid in Millipore water and the organic mobile phase B was 0.1% formic acid in methanol. The flow rate was kept constant at 0.600 mL/min for the entirety of the run. The total run time for the method was 11 minutes.

Results: A linear dynamic range of 5.0-200.0 ng/mL was established for all four analytes. Based on the established calibration model, the LOD ranged from 1.92-3.31 ng/mL for and the LOQ for all analytes was 5 ng/mL. Most analytes displayed acceptable bias of ±20%. Precision was analyzed concurrently and determined to be within for ±20% for all analytes. All analytes were determined to be free from significant carryover. No matrix interference peaks were observed in blank urine samples which fell within 2% of a known analyte retention time and had a signal intensity greater than the calculated LOD.

Conclusions: B-One™ and BGTurbo® from Finden by Kura are user-friendly, with explicit instructions for enzyme hydrolysis mix formulation and incubation steps, thus facilitating the integration of an enzymatic hydrolysis in sample preparation.

Table 1. Enzymatic Hydrolysis Conditions

Enzyme	Temperature (°C)	pH	Incubation Time (mins)
Kura Biotech – B-One™ Enzymatic Activity: 12,000 PS-U/mL	~22 (room temperature)	6.8	5
Kura Biotech – BGTurbo® Enzymatic Activity: 200,000 U/mL	55	6.8	10
Sigma – Fast β-Glucuronidase, Recombinant from limpets (*P. vulgata*) Enzymatic Activity: 300,000-400,000 U/mL	70	5.2	15

Table 2. Hydrolysis Recovery Data (BGTurbo®)

Analyte	Average Hydrolysis Recovery
Morphine	40.6193909
Codeine	32.31761758
Dihydrocodeine	42.32163647
Hydromorphone	71.20118315

Table 3. Hydrolysis Recovery Data (B-One™)

Analyte	Average Hydrolysis Recovery
Morphine	62.7053424
Codeine	70.29553515
Dihydrocodeine	71.26788322
Hydromorphone	94.38685007

Table 4. Hydrolysis Recovery Data (Sigma)

Analyte	Average Hydrolysis Recovery
Morphine	95.31406112
Codeine	13.66558141
Dihydrocodeine	6.39794972
Hydromorphone	126.6236062

POSTER

P-023: Pivot Chart Primer: Experiencing the Joy of Excel's Hidden Gem to Critically Evaluate Forensic Toxicology Productivity

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Background/Introduction: Objectively analyzing productivity and administrative metrics is a critical need for management in forensic science. Historically, ADFS has extracted case and productivity data from Porter Lee's BEAST Laboratory Information Management System (LIMS) using Crystal Reports. The data are then exported into Excel spreadsheets and further analyzed using the filter function and constructing graphs for each data set individually. Constructing graphs and analyzing data can be time consuming when a number of different metrics are to be analyzed. Instead of the traditional method, Microsoft Excel's pivot chart/table feature can be used to minimize time while expanding the perspective and capability for trend analysis. Microsoft Excel's pivot chart/table feature is easily accessible and allows one to analyze data from difference views with a simple operation at no additional cost.

Objectives: To develop an effective tool to critically analyze administrative and productivity statistics, evaluate analyst performance, and monitor trends in forensic casework.

Methods: ADFS data were extracted from Porter Lee's BEAST LIMS and Crystal Reports, then exported into Microsoft Excel spread-sheets. The following parameters for calendar year 2020 were used: tasks completed per analyst, case type, task type, and date of completion. ADFS Toxicology Section currently employs an individual task based system as a mechanism for assigning and tracking the individual components of casework such as login, individual extractions, data-analysis, or drafting a report. Case types include homicide (01), death non-homicide (27), other criminals (26), drivers (24), DUI (21), and rape/sexual assault (02/17). A spreadsheet tab was created for each aspect of data and a respective pivot chart/table was generated. The charts and tables were organized in a dashboard tab and slicers (interactive filtering feature) were created to adjust the view of the linked charts and tables.

Results: An Excel dashboard was created utilizing multiple linked pivot charts and slicers to analyze the productivity of ADFS's Toxicology section which allowed for efficient periodic updating without the need to recreate charts and graphs. Features include the ability to adjust preferences based on analyst, date of task completion, type of task, and case type. This flexibility allows the manager to add, rearrange, or remove fields to visualize key areas of interest such as extractions, data analysis, or drafted reports per scientist. With minimal adjustments in selections using slicers, one can compare performance of scientists working on-site versus off-site (tele-working), or the productivity of five eight-hour shifts versus four ten-hour days. The created pivot chart also allows the opportunity to monitor demand for specific instrumentation such as GC/MS vs. LC/MS/MS.

Conclusion/Discussion: After charting trends, management was able to position players in the laboratory based on their strengths. Conclusions derived create objective evidence of performance used in annual evaluations or in establishing individual and team month-ly productivity goals. The insight provided has been key in making annual budget recommendations to obtain equipment, instrumentation, and DUI collection kits for subsequent years. The ability to forecast productivity has also aided in the prioritization and timely completion of medical examiner cases to meet National Association of Medical Examiners (NAME) standards. Excel's pivot chart/pivot table feature is of useful utility in managing a Forensic Toxicology laboratory allowing for the flexible interactive analysis of productivity statistics.

POSTER

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P-024: Enrichment of Hemoglobin Covalently Adducted at β⁹³Cys by Reactive Xenobiotics as Potential Biomarkers of Drug Exposure

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Background/Introduction: Hemoglobin (Hb) is an abundant blood protein that contains unbound cysteine thiols. These nucleophilic moieties can covalently adduct to reactive electrophilic species. In particular, the Hb **β⁹³Cys** residue has been shown to be highly reactive with electrophilic xenobiotics. To date, the use of covalent protein modifications as biomarkers for long-term illicit drug exposure has not been extensively explored. Identifying and characterizing covalent Hb adducts has been described as "finding a needle in a haystack," as the abundance of covalently modified protein is very low compared to unmodified protein. One solution is an enrichment method that could selectively remove unadducted Hb, allowing for greater sensitivity in the analysis of modified protein.

Objectives: The objective of this work was to adapt a published enrichment assay for adducted serum albumin for use with Hb to increase the sensitivity for analysis of covalent adducts at the **β⁹³Cys** position. For proof of concept, N-ethylmaleimide (NEM) and acetaminophen (APAP) were used as test compounds for thiol binding.

Methods: An aliquot of 2.5 mg/mL human Hb was incubated with 150 mM NEM in ammonium bicarbonate buffer (AmBic) at pH 7.4. After centrifugation in a 3 kDa MW cutoff filter, covalently adducted product (Hb-NEM) was collected and diluted to a concentration of 0.4 mg/mL. Test samples were generated by mixing control Hb and Hb-NEM in molar ratios of ~600:1, ~6,000:1, and ~60,000:1. Hb mixture was added to an equal part thiol affinity resin (Sepharose 4B in 0.5 M NaCl, 100 mM Tris-HCl, pH 7.4) and incubated for 18 h to trap unadducted Hb. After centrifugation, supernatant containing enriched Hb-NEM was collected and buffer exchanged into fresh AmBic. Aliquots were taken for whole protein MS analysis and tryptic digestion for peptide analysis. An Agilent 1290/6530 LC-QTOF MS was used for proteomic analyses. BioConfirm and Protein Prospector software were used to process whole protein and tryptic peptide MS and MS/MS spectral data. For APAP, an *in vitro* enzymatic trapping assay prior to enrichment was used to test covalent adduct binding of APAP reactive metabolites (RM). An aliquot of 2.5 mg/mL Hb was incubated with 500 µM APAP in a human liver microsomal enzyme system for 6 h. After centrifugation in a 3 kDa filter, Hb was subjected to the enrichment assay to isolate Hb-APAP and proteomic analysis was performed as described for NEM.

Results: Whole protein spectra gave initial confirmation of covalent protein modification by providing a mass shift for the specific modification of interest. Tryptic peptide mapping was then used to determine the specificity of the covalent binding site. Data for unadducted Hb and Hb-NEM mixtures identified a mass shift of +125.05 Da, representing a single modification by NEM. MS and MS/MS data confirmed that modification was at the *β*⁹³Cys moiety and that MS/MS fragments contained the covalent NEM adduct. Data for APAP indicated a mass shift of +149.68 Da, representing a single modification by the APAP RM NAPQI. MS and MS/MS data confirmed covalent modification at the *β*⁹³Cys of Hb with a NAPQI adduct.

Conclusion/Discussion: A major challenge for analysis of covalent protein modifications by reactive metabolites of drugs of abuse is the abundance of adducted protein species are typically extremely low. With the use of an enrichment assay, a greater sensitivity in the detection of Hb adducted species can be accomplished, making this alternative biomarker a potential candidate for long-term retrospective drug exposure monitoring.

POSTER

P-025: Validation of an automated sample preparation technique for the comprehensive screening of biological matrices using liquid chromatography time-of-flight mass spectrometry

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Background/Introduction: Forensic toxicology analyses rely heavily on the results obtained from qualitative screening methods. Given the limitations of these methods, an individual case sample tends to require multiple analyses to obtain comprehensive qualitative information. In conjunction with the utilization of multiple analytical methods, sample preparation can be laborious and time consuming. Automated sample preparation and liquid chromatography-high resolution mass spectrometry (LC-HRMS) can be employed to comprehensively evaluate biological samples for toxicologically significant drugs and metabolites using a single analytical technique. Over a two year period, the Virginia Department of Forensic Science Toxicology Section observed a 32% increase in the number of qualitative and quantitative drugs reported. Given the number of qualitative and quantitative drugs reported annually and the variety of drugs evaluated, it is essential that a screening technique be comprehensive, efficient, and robust to decrease laboratory turnaround times without limiting the scope of testing.

Objectives: To develop and validate a fully automated solid phase extraction (SPE) sample preparation technique to be used for the comprehensive screening of over 250 compounds in antemortem and postmortem biological matrices using LC-HRMS. In addition to the validation, evaluate a false positive/negative rate associated with the comprehensive screen by screening and confirming over 400 antemortem blood samples.

Methods: The validation is in accordance with ANSI/ASB Standard 036 Standard Practices for Method Validation in Forensic Toxicology. Additional method validation experiments were conducted to verify the functionality of the automated sample preparation procedure in addition to the establishment of acceptance criteria including the utilization of mass match, mass accuracy, mass spacing, and mass abundance scoring for LC-HRMS analysis. The SPE utilizes 0.5 mL of biological matrix that is extracted and eluted into two fractions for analysis. The extraction includes column conditioning, addition of the sample, and column washing. After the columns are washed with acetic acid, columns are dried and samples are eluted with 3.0 mL of hexane:ethyl acetate (9:1) followed by 3.0 mL of isopropanol. The columns are further washed with methanol and dried prior to sample elution with 3.0 mL of 78:20:2 methylene chloride:isopropanol:ammonium hydroxide. Both eluate fractions are collected individually, dried down, and reconstituted prior to analysis using LC-HRMS. To further evaluate the method, over 400 antemortem blood specimens were analyzed using the LC-HRMS method and the results confirmed utilizing other previously validated confirmation and quantitation techniques. Through validation and the analysis of authentic biological specimens, the fully automated SPE extraction is intended to offer a streamlined approach to the comprehensive screening of biological matrices within forensic toxicology laboratories.

Results: During validation of the automated sample preparation, cross contamination was observed and thoroughly investigated to mitigate potential sources. Additionally, extensive enhancements to the LC-HRMS software were performed to ensure data robustness. Within the analytical method, a low threshold and high threshold sample was evaluated for each compound. Given the number of compounds and the breadth of concentrations observed for various compounds in antemortem and postmortem toxicology, ten different concentration ranges were established. These concentrations stressed the data analysis software and its abilities to evaluate saturated peaks within a chromatogram. Method validation experiments were performed for all analytes within the comprehensive screen. Although some compounds did not pass the predetermined validation acceptance criteria, these compounds were determined insignificant in comparison to other compounds within the method.

Conclusion/Discussion: The automated sample preparation procedure in conjunction with LC-HRMS analysis provides a unique approach to sample preparation within forensic toxicology analysis. The identification of individual compounds in conjunction with the potential time savings associated with automated sample preparation has the potential to significantly impact the workflow associated with forensic toxicology analyses.

P-026: Assessment of oxycodone and metabolite concentrations in urine and oral fluid in patients in prescription drug monitoring programs

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Background/Introduction: Oxycodone [OXYC], a semi-synthetic derivative of thebaine, is used as an analgesic for the relief of moderate to severe pain. OXYC undergoes cytochrome P450 [CYP] dependent O-and N- demethylation to form the active metabolite oxymorphone [OXYM] by CYP 2D6 and the relatively inactive noroxycodone [NOXYC] by CYP3A. Measurement of these compounds is clinically useful to assist in the evaluation of adherence to a treatment regimen.

Objectives: This retrospective study was performed to evaluate urine and oral fluid [OF] concentrations of OXYC and metabolites from a population in New England, USA. The objective of the study was to assess the prevalence of parent drug and metabolites and compare and contrast findings in urine and OF.

Methods: Random urine and OF specimens from April 1, 2020 to March 31, 2021, were subject to identification and quantitation of OXYC, OXYM, NOXYC by liquid chromatography-tandem mass spectrometry [LC-MS/MS]. Hydrolyzed urine specimens containing deuterated internal standards were prepared by solid phase extraction followed by LC-MS/MS analysis with chromatographic resolution using Phenomenex Kinetex Biphenyl, 50 × 3.0 mm, 2.6 mcm, 100Å column coupled to a Sciex 4500 mass spectrometer . The clinical reporting range [CRR] for urine was 50-10,000 ng/mL. OF specimens were also analyzed by LC-MS/MS. The CRR for OF was 2.5-250 ng/mL.

Results:

URINE

During the study period, a total of 10,491 specimens were subject to direct quantitative analysis. At least one analyte was detected in 2,582 specimens. Several specimens had drug concentrations >CRR. Table 1 shows the number of specimens in which OXYC and/or its metabolites were detected and their concentrations.

Table 1 Distribution of OXYC and its Metabolites in Urine and Quantitative Values (ng/mL) within CRR

Drug/metabolite	N	N within CRR	Range	Mean± SD	Median
OXYC	2220	1997	50-9991	1924±2154	1067
OXYM	2341	2194	50-9994	1569±1998	741
NOXYC	2461	2091	50-9992	2363±2405	1456

All three analytes were detected in 2,063 [79.9%] specimens with 46 [1.8%] OXYC only, 72 [2.8%] OXYM only, and 87 [3.4%] NOXYC only. OXYC+NOXYC combination was found in 108 [4.2%], OXYC+OXYM combination was found in 3 [0.1%], and OXYM+NOXYC combination was found in 203 [7.9%] of specimens.

ORAL FLUID

During the study period a total of 16,647 specimens were subject to analysis. At least one analyte was detected in 2,073 specimens. Several specimens had drug concentrations >CRR. Table 2 shows the number of specimens in which OXYC and/or its metabolites were detected and their concentrations.

Table 2 Distribution of OXYC and its Metabolites in OF and Quantitative Values (ng/mL) within CRR

Drug/metabolite	N	N within CRR	Range	Mean± SD	Median
OXYC	1763	1086	2.5-249.9	77.5±71.6	55.6
OXYM	622	612	2.5-236.3	14.9±27.0	5.7
NOXYC	1532	1448	2.5-248.7	43.8±49.4	24.3

All three analytes were detected in 322 [15.5%] specimens with 241 [11.6%] OXYC only, 295 [14.2%] OXYM only, and 15 [0.7%] NOXYC only. OXYC+NOXYC combination was found in 1,195 [57.6%], OXYC+OXYM combination was found in 5 [0.2 %], and OXYM+NOXYC combination was not found in any [0%] specimens.

Conclusion/Discussion: This study demonstrated that OXYC and metabolites in urine were found throughout and above the CRR. All three analytes were detected in approximately 80% of specimens, with low prevalence of single analyte samples. For drug concentrations within the CRR Mean± SD values were similar for each compound with more variability in median results. The OF results showed a different pattern with <16% of specimens containing all three analytes with OXYC+NOXYC combination most prevalent. This study demonstrated that the measurement of NOXYC plays an important role in detecting oxycodone use and should be a component of an oxycodone panel.

POSTER

P-027: Development and Validation of a GC-FID Method for 20 Different Acidic and Neutral Cannabinoids

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Background/Introduction: *Cannabis sativa* is one of the oldest studied plants in the world. It has been utilized for a variety of purposes, including food, textile, and medicinal and therapeutic uses for the past 5,000 years. For decades, *Cannabis sativa* had been illegal to sell or consume around the world, including the United States; however, with the changes in the legal status of cannabis in many states, cannabis products are currently flooding the illicit market. Previously, we developed and validated a GC-FID method for the analysis of 13 acidic and neutral cannabinoids in *C. sativa* extracts, and we are now expanding the method to cover additional cannabinoids in this study.

Objectives: The objective of this method is to expand a previously validated GC-FID procedure to cover an additional 7 acidic and neutral cannabinoids, giving a cannabinoid profile of different cannabis varieties, and commercial products, for 20 cannabinoids.

Methods: Previously, a GC-FID method was developed and validated for the analysis of 13 cannabinoids, namely Δ^9-tetrahydrocannabivarin (THCV), cannabichromene (CBC), cannabidiol (CBD), (-)-*trans*-Δ^8-tetrahydrocannabinol (Δ^8-THC), (-)-*trans*-Δ^9-tetrahydrocannabinol (Δ^9-THC), cannabigerol (CBG), cannabinol (CBN), cannabidiolic acid (CBDA), cannabigerolic acid (CBGA), Δ^9-tetrahydrocannabinolic acid-A (THCAA), cannabidivarin (CBDV), cannabicyclol (CBL), and cannabidivarinic acid (CBDVA) following the ICH validation protocol[1]. The seven additional cannabinoids in the current GC-FID method are cannabielsoin (CBE), cananbitriol (CBT), cannabichromenic acid (CBCA), cannabielsoic acid (CBEA), cannabicyclolic acid (CBLA), cannabinolic acid (CBNA), and **Δ^9-tetrahydrocannabivarinic acid (THCVA)**. The column used for the analysis was a DB-1MS. The method was validated following the ICH Guidelines.

Results: The limit of detection (LOD) for all 20 acidic and neutral cannabinoids was determined to be as low as 1 ppm. For the 3 controls used (1.25, 2.5, and 5 ppm), the accuracy ranged from 93% - 98%, 96% - 99%, and 96% - 99% respectively. The precision ranged from 93% - 98%, 97% - 99%, and 96% - 99% respectively. The correlation coefficient (r^2) for the linearity curves ranged from 0.9938 – 1.0 for all 20 cannabinoids.

Conclusion/Discussion: The developed method is simple, sensitive, and reproducible for the quantitation of 20 different acidic and neutral cannabinoids in cannabis biomass and cannabis based commercial products. The results of this analysis will be discussed in details.

[1]Ibrahim, E. A., Gul, W., Gul, S. W., et al. (2018). Determination of acid and neutral cannabinoids in extracts of different strains of Cannabis sativa using GC-FID. *Planta medica*, *84*(04), 250-259.

POSTER

P-028: High-Capacity Screening for Compounds of Forensic Toxicological Interest Using a Standardized LC-MS/MS System and Method

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Background/Introduction: The universe of compounds that need to be screened for forensic purposes is enormous and increases almost daily. Maintaining the capacity to detect even a significant fraction of this universe can be both technologically and economically prohibitive. Forensic laboratories need the capacity to analyze large numbers of compounds in a single run with consistent results. This work presents a proven, standardized liquid chromatograph-mass spectrometer (LC-MS/MS) system, method, and workstream capable of consistently and reliably screening select panels from a database of over 1100 forensic analytes in a single chromatographic run. While this workstream has previously been demonstrated on high-resolution, accurate-mass mass spectrometers, this work demonstrates the ability to achieve similar results on hardware accessible to more toxicology laboratories, a triple quadrupole mass spectrometer.

Objectives: The objectives of this work were to: A) develop a standardized LC-MS/MS configuration and method capable of quickly and reliably screening large panels of compounds of forensic interest, B) create a method that, when used on any identical LC-MS/MS system, would achieve consistent analytical results, and C) to achieve all of this on a widely familiar and economical MS platform. To test the method's effectiveness, 101 representative compounds—selected to cover different drug classes, retention times, hydrophobicities, and ionization polarities—were analyzed in a urine matrix in a single analytical run.

Methods: The developed system consisted of a Vanquish Flex liquid chromatograph and TSQ Quantis triple quadrupole mass spectrometer, TraceFinder data acquisition and analysis software with an 1100+ compound database, and operating parameters (method). The LC included specific tubing lengths and an Accucore phenyl-hexyl 2.6 μM, 100 x 2.1 mm HPLC column. Mobile phases were water (A) and methanol:acetonitrile (1:1) (B), both with 2 mM ammonium formate and 0.1% formic acid. Gradient length was 15.5 minutes.

MS was equipped with a heated electrospray ionization source and set at parameters below:

Parameter	Value
Spray Voltage (+/-)	3500/2500 V
Ion Transfer Tube Temp	325 C
Vaporizer Temp	350 C
Gasses	
• Sheath	50 Arb
• Aux	10 Arb
• Sweep	1 Arb

The TraceFinder Compound Database contained optimized selected reaction monitoring (SRM) transitions, RF settings, and retention times for each target compound. This database was used to generate the SRM table for the MS. The SRM transitions for a given compound were monitored in a one-minute window around the nominal retention time. To verify performance, 101 representative compounds of forensic toxicological interest were analyzed in a urine matrix using parameters taken directly from the database. The compounds included opiates, benzodiazepines, anti-depressants, amphetamines, synthetic cannabinoids, cathinones, and other novel psychoactive substances. All compounds were analyzed at 14 concentration levels from 0.1 to 2000 ng/mL. Samples were processed by addition of internal standards followed by 20-fold dilution with water before injection.

Results: All 101 compounds were successfully detected in a single chromatographic run. Although designed primarily as a screening method, all 101 compounds were also accurately quantified based on developed calibration curves. Limits of detection, defined as detection of at least one MS-MS transition within the retention time window, ranged from 0.1 to 100 ng/mL. Limits of quantitation, defined as a back-calculated concentration within 30% of nominal on the calculated calibration curve, ranged from 0.1 to 200 ng/mL.

Conclusion/Discussion: Previous work demonstrated the ability to screen over 1100 compounds of forensic interest in a single analysis on a high-resolution accurate-mass LC-MS system. In this work, we implemented a method capable of screening very large panels from the same 1100 compound list on a triple quadrupole LC-MS/MS system. Implementing this high-capacity screening method on a more economical MS platform makes this powerful capability accessible to more forensic toxicological laboratories.

POSTER

P-029: Method Validation for the Simultaneous Quantification of 17 Commonly Encountered Central Nervous System Depressants (CNSD) in Whole Blood by LC-MS/MS

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Background/Introduction: In 2020, a total of 1,190 cases were submitted by the Rhode Island Center for the Office of State Medical Examiners for analytical testing by the Rhode Island Forensic Toxicology Laboratory (RIFTL). These submissions included 141 cases (13.0%) that were positive for central nervous system depressants (CNSD) with a majority (129) related to benzodiazepines (11.9%). This represented the third most commonly encountered compound class, trailing only opioids and stimulants. Liquid chromatography/ tandem mass spectrometry (LC-MS/MS) was employed to validate a 17-analyte panel for in-house CNSD analysis to expand the scope of CNSD quantified in post-mortem specimens and reduce the time to obtain laboratory results. This method also evaluates presence/ absence (statutory requirement) of these same compounds in specimens submitted for approximately 200 impaired driving cases submitted to RIFTL annually.

Objective: Develop and validate a LC-MS/MS method that allows for a single sample preparation and quantitation of diazepam, nor-diazepam, oxazepam, temazepam, chlordiazepoxide, lorazepam, clonazepam, 7-aminoclonazepam, alprazolam, alpha-hydroxyalpra-zolam, midazolam, flurazepam, desalkylflurazepam, eszopiclone/zopiclone, zolpidem, carisoprodol, and meprobamate in whole blood.

Methods: 800 μL of 0.1 M zinc sulfate and ammonium acetate solution was added to a microcentrifuge tube followed by the addition of a 100 μL mixture of complementary deuterated internal standard (IS). 200 μL whole blood aliquots of samples, controls, or calibrators were then added and vortexed 5-10 seconds. Samples were then vortexed for 30 seconds prior to centrifugation at 12,500 RPM for 10 minutes. The supernatant was then diluted with 900 μL of 4% phosphoric acid. Pretreated samples were loaded on to a Waters Oasis MCX™ 96-well solid-phase extraction (SPE) plate and decanted with a 96-well positive pressure manifold. Samples were washed with 500 μL of 0.1% formic acid (FA) followed by 10% methanol, dried at high positive pressure for 5 minutes and eluted with acetonitrile: methanol (60:40) containing 5% strong ammonia. Samples were evaporated to dryness and reconstituted with 100 μL of water: acetonitrile: FA (97:2:1) for analysis. LC-MS/MS analysis was performed using a Waters® ACQUITY UPLC H-Class LC system coupled to a Waters® Xevo TQD. Mobile phases were: 0.1% FA in Milli-Q water (Solvent A) and 0.1% FA in acetonitrile (Solvent B). The optimized flow rate was 0.4 mL/min using gradient elution with initial conditions of 98:2 (Solvent A: Solvent B) increased to 5:95 over 8.5 minutes then returned to 98:2. The system re-equilibrated for 2.0 minutes with an entire cycle time of 10.5 minutes. A Waters ACQUITY™ BEH C18 1.7μm x 2.1 x 100mm column was maintained at 40°C. Autosampler temperature was maintained at 5°C. Injection volume was 5 μL and the needle was purged with methanol: water (50:50) between injections and washed with water: methanol: acetonitrile: isopropanol (1:1:1:1). Two transitions were monitored for each analyte and internal standard. Validation was performed using ANSI/ASB Standard Practices for Method Validation in Forensic Toxicology, First Edition (2019) and the RI Forensic Toxicology laboratory guidelines.

Results: All analyte data was analyzed with RStudio® to determine appropriate calibration model and weighting. LOD's ranged from 2.5-5 ng/mL and LOQ's ranged from 5-10 ng/mL. Percent bias and %CV for all analytes was within acceptable range of ± 20%. Analytes in samples previously identified quantitatively by other methods demonstrated 100% concordance with this method. Matrix and ionization effects were noted and impacts on LOD, LOQ, and bias evaluated. These were controlled for using matrix-matched controls and calibrators with deuterated IS. No significant carryover or interference from other drugs was observed. Extracts were stable 1 day and preparations of a 1:2 dilution successfully quantitated.

Conclusion/Discussion: A method for the simultaneous identification and quantitation of 17 CNS compounds in whole blood was successfully validated using SPE and LC-MS/MS.

P-030: Liquid Chromatography Tandem Mass Spectrometry for the Identification and Quantitation of Kratom Alkaloids

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Background/Introduction: Kratom (Mitragyna speciosa) is a psychoactive natural product that presents several analytical challenges. Most reported analytical methods have focused on the detection of mitragynine (MG) as it has been reported to be 13 times more potent than morphine[1]. More recently there has been concern over the toxicity of mitragynine, as there have been an increase in toxicology casework with driving under the influence of drugs and death investigation cases. Over 20 related compounds including structural isomers and diastereomers are present in Kratom. We discuss here the chromatographic separation and identification of MG and its two diastereomers [speciociliatine (SC) and speciogynine (SG)] along with paynantheine (PY) using liquid chromatography tandem mass spectrometry (LC-MS/MS). Mitragynine-d3 (MG-d3) was used as an internal standard for the calibration curve. The LC-MS/MS analysis was performed on a LCMS-8060NX triple quadrupole instrument due to its higher sensitivity and greater robustness for samples in complex matrices. This is particularly of importance for forensic samples (blood, urine, tissues) where matrix can have a significant influence on the robustness and throughput of the assay.

Objectives: This work aimed to separate, identify, and quantify four Kratom alkaloids (mitragynine, speciociliatine, speciogynine, and paynantheine) using LC-MS/MS in neat samples.

Results:

	MG	SC	SG	PY
Range (ng/mL)	0.03-485	0.03-485	0.03-485	0.03-485
r^2	0.996	0.991	0.995	0.995
Weighting	$1/X^2$	$1/X^2$	$1/X^2$	$1/X^2$
LOQ (ng/mL)	0.03	0.03	0.03	0.03
LOD (ng/mL)	0.03	0.03	0.006	0.006
Accuracy (%)	93.8-108.2	89.3-117.8	91.5-109.1	92.4-110.5
Carryover	None observed	None observed	None observed	None observed
Precision (%RSD n=3)	0.4-24.5	2.5-13.3	0.6-19.8	0.3-11.0

Methods: The LCMS-8060NX triple quadrupole mass spectrometer was coupled with a Nexera UHPLC system. The interface parameters were optimized by flow injection analysis (FIA).. The MRM optimization was performed for all the standards including the internal standard with the MRM optimization tool in LabSolutions using FIA. The chromatographic separation was performed on Ascentis Express C18 column (100x3mm, 2.7 µm) held at 35ºC. The mobile phase was operated at a flow rate of 0.4 mL/min and the duration of gradient run was 10 minutes. A standard stock mix was prepared with all the four analytes in methanol. The stock solution was then diluted in 0.03-485 ng/mL range with methanol to obtain a seven—point calibration curve (0.03, 0.16, 0.8, 3.9, 19.4, 97 and 485 ng/mL). The internal standard (IS) MG-d3 was added at 100 ng/mL concentration at all the calibration points. The calibrators were injected in triplicate at an injection volume of 0.5 µL. Linear calibration curves with IS along with percent accuracy calculations were generated using LabSolutions. The percent accuracy for each calibrant was determined based off of experimental vs. theoretical concentration. The average % accuracy and corresponding %RSD for triplicate injections was calculated. The reported precision includes the range for all calibrant levels.

Conclusion/Discussion: A rapid and reliable identification and quantitation method for the analysis of four Kratom alkaloids MG, PY, SC, and ST was developed using LCMS-8060NX instrument. The reported method employs baseline chromatographic separation of all four alkaloids for identification and quantitation. Sensitivity (15.5 fg on column), linearity (r^2>0.99) accuracy (89.9-117.8% range) and precision (%RSD 0.3-24.5) were observed for all four alkaloids. This rapid 10-minute LC-MS/MS method can easily be deployed in any forensic laboratory looking for a high throughput and robust analytical method for Kratom alkaloid analysis.

References: [1] *J. of Anal. Tox*;, 2019; 43:615-629

POSTER

P-031: Quantitative Analysis of Illicit Drugs of Abuse in Human Whole Blood by LC-MS/MS

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Background/Introduction: The escalation of many forms of illicit drugs of abuse in forensic toxicology casework necessitates development of a comprehensive analytical method that requires minimum sample preparation. Whole blood has been the biological specimen of choice for many forensic toxicologists in accidental death cases, especially in those where drug use is associated with the cause of death. Due to many constraints like budget and resource limitation, there is a high demand for a cost effective, efficient method of analysis for forensic toxicology laboratories. In this communication, we demonstrate a fast and effective sample preparation method that targets a broad range of pain management and illicit drugs in whole blood, utilizing Phree™ Phospholipid Removal (PLR) products. Phree allows for a simplified sample clean up that is efficient and reliable for analysis of forty compounds, comprising of opiates (both natural and synthetic), amphetamines, benzodiazepines, and other illicit drugs (fentanyl analogs, PCP and cocaine metabolites). The effective clean up technique lends itself useful for detection by LC-MS/MS utilizing a SCIEX 4500 Triple Quad™ mass spectrometer (QQQ-MS) equipped with ESI source.

Objectives: The objective of this work is to develop a fast and reliable quantitation method for the detection of forty drugs of abuse in whole blood that is cost effective.

Methods: The sample is pre-treated by adding 50 μL of 5% $ZnSO_4$ solution to 200 μL of whole blood. Next, the sample undergoes protein precipitation using 600 uL chilled (0 to -20°C) 95:5 acetonitrile: methanol. The supernatant of the precipitated filtrate is then acidified before being introduced to a Phree PLR 96-well plate. The LC-MS/MS method utilized a Kinetex® 2.6 um, Biphenyl core-shell 50x3.0 mm LC column with a flow rate at 500 mL/min. Mobile phase A and B were consisted of water and methanol respectively, both acidified with 0.1% formic acid. A gradient from 15% to 95% of mobile phase B was applied in 3.5 minutes, followed by a hold for 1.5 minutes at 95% B, to allow separation of all 40 analytes in this drug panel. The analytical detection was carried out on a SCIEX 4500 QQQ-MS equipped with ESI source, operated under positive ionization mode.

Results: The absolute recovery yield obtained from the Phree PLR cleanup step ranged from 76-114% with a % CV value ≤15%. The recovery data obtained from a simple protein precipitation in parallel was compared and found equivalent. However, Phree PLR extraction method was far superior in terms of sample clean-up that effectively removed more than 95% of the phospholipids, resulted from a simple protein precipitation in whole blood sample. Calibration curves were constructed in spiked whole blood over a wide dynamic range expanding from 0.075 ng/mL to 1500 ng/mL concentration level, depending on the unique cutoff requirement values of the analytes of interest. A quadratic fit with 1/x weighting factor demonstrates linearity for all analytes displaying a linear regression value (R^2) ranging ≥0.996.

Table 1. List of drug panel

Classes	Analytes
Benzodiazepines	Alprazolam
	Clonazepam
	Diazepam
	Flunitrazepam
	Flurazepam
	Lorazepam
	Midazolam
	Nordiazepam
	Oxazepam
	Temazepam
Opiates	Codeine
	Hydrocodone
	Oxycodone
	Hydromorphone
	Morphine
	Oxymorphone

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	Methadone
	EDDP
	6-MAM
	Fentanyl
	Norfentanyl
	Meperidine
	Normeperidine
	Naloxone
	Norpropoxyphene
	Propoxyphene
	Sufentanil
	Naltrexone
Amphetamines	Amphetamines
	Methamphetamines
	MDMA
	MDA
	MDEA
Analgesics	Meprobamate
	Tramadol
	Carisoprodol
	Buprenorphine
	Norbuprenorphine
Others	Phencyclidine
	Benzoylecgonine

Conclusion/Discussion: The fast and effective Phree PLR sample clean up in a 96-well plate format, meets the demand of a large accrual of samples while integrated with the Kinetex core-shell Biphenyl LC-MS/MS method. The developed analytical method successfully overcomes matrix dependent challenges to quantitate this drugs of abuse panel reliably and efficiently.

POSTER

P-032: Implementing Quantitative Drug Screening Using High-Resolution Mass Spectrometry

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Background/Introduction: Drug abuse remains a serious social and economic issue worldwide as drugs continue to pose a threat to social stability and economic development. As the emergence of novel substances continues to pose public health and safety problems, drug testing remains one of the most effective measures for global drug control. The use of high-resolution mass spectrometry (HRMS) for drug testing has enabled toxicologists to rapidly screen the presence of these drugs by acquiring their complete chemical profile.

Objectives: The objective of this study is to develop a rapid and comprehensive drug screening workflow for the analysis of urine samples using high-resolution mass spectrometry.

Methods: A total of 39 drugs commonly screened were selected for this panel. Three calibrator solutions ranging from 5 to 5000 ng/mL were prepared. A dilute-and-shoot sample preparation method was used for the detection of the 39 drugs in urine. Blank urine samples were fortified with the three calibrator solutions and diluted 10-fold with a solution of methanol: water (10:90, v/v). Fortified urine samples were thoroughly vortexed, centrifuged for 10 min at 12,000 rpm and the clear supernatant was transferred to autosampler vials for analysis. Analytes were chromatographically separated at 45˚C using a Phenomenex Phenyl-Hexyl column (50 × 2.1 mm, 2.6 µm, 00B-4495-E0). Mobile phases used consisted of HPLC grade water with 0.1% formic acid (mobile phase A) and methanol with 0.1% formic acid (mobile phase B). The injection volume was 10 µL and the total LC runtime was 8.5 minutes. MS and MS/MS data were collected for each sample using the SCIEX X500R QTOF System. Information Dependent Acquisition (IDA) was used in positive ion mode. MRM^{HR} workflow with the Apply TOF start/stop mass feature activated was used in negative ion mode.

Results: The quantitative performance of the assay was investigated by injecting a series of diluted urine samples at three concentration levels ranging from LOD to 10x LOD for each of the 39 drugs used in this panel. The three-point calibration curves showed excellent correlation and linearity with R^2 values above 0.97 for all the 39 drugs targeted in this study. The reproducibility and robustness of the data was also investigated. Four confidence criteria were used for confident identification of the 39 drugs targeted in this study. They included mass accuracy, RT, % difference in isotope ratio, MS/MS library matching and were assigned a percentage to generate a combined score. Overall, the assay showed excellent reproducibility (ranging from 0.13 and 12.39%) and accuracy (ranging from 81.72 and 112.78%), proving the robustness of the developed workflow. The overall robustness of the workflow was further investigated by analyzing a series of urine case samples. Results from the analysis of one of the urine case samples showed successful detection of hydrocodone, hydromorphone, lorazepam and norhydrocodone at concentration of 63.07, 99.08, 574.20 and 311.10 ng/mL, respectively. The library matching scores (>97%) together with the excellent calculated combined scores (>95%) for the four detected analytes provides a confident metric for the accurate identification of the drugs in this urine case sample.

Conclusion/Discussion: A rapid and comprehensive drug screening workflow for the analysis of urine case samples was successfully developed using the SCIEX X500R QTOF System. A simple dilute-and-shoot sample preparation method combined with a robust data acquisition strategy enabled collection of high-quality MS/MS spectra, allowing reliable compound quantitation and identification through spectral library matching. Positive drug identification was confirmed using the confidence criteria. The information that can be inferred from the results offer a valuable insight on drug intake, providing health professionals a clearer picture of the long term use of these substances.

POSTER

P-033: Multi-panel detection of drugs and drug metabolites in hair samples using a comprehensive extraction method

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Background/Introduction: The ability to accurately identify the presence of a variety of drugs and drug metabolites in biological specimens is a critical aspect to any forensic and clinical toxicology investigation as it provides a comprehensive picture of past drug exposure towards xenobiotics, a history of the non-endogenous substances in the human body. In recent years, hair testing has gained considerable attention as a method enabling the determination of recent past drug use as well as long-term drug use through segmental analysis.

Objectives: The objective of this study was to develop a comprehensive workflow for the detection of a wide range of drugs and drug metabolites in hair.

Methods: A total of 77 compounds were included in the novel psychoactive substance (NPS) panel. The drugs of abuse (DOA) panel consisted of 23 compounds and the third panel consisted of Ethyl glucuronide (EtG) only. Hair samples were washed thoroughly with methanol for 5 minutes then diethyl ether for another 5 minutes, dried and spiked with the various drug calibrator mixtures. 500 µL of a proprietary aqueous extracting solution was added to the spiked hair samples, gently vortexed, centrifuged at 5,000 rpm for 1 minute and incubated for 60 minutes at 100˚C. Following the incubation step, the liquid extract was transferred to a new tube and centrifuged at 5,000 rpm for 5 minutes. The organic phase was transferred to a sample vial for analysis. Analytes were chromatographically separated at 50˚C using a Phenomenex Synergi 2.5 µm Hydro-RP column (50 x 3 mm, 2.5 µm, P/N 00D-4387-Y0). Mobile phases used consisted of HPLC grade water with 0.1% formic acid (mobile phase A) and acetonitrile with 0.1% formic acid (mobile phase B). Three separate gradients, flow rates and injection volumes were were used for each of the LC methods. MS data were collected in positive mode on the SCIEX QTRAP 6500+ System. Three separate acquisition methods were created using the Scheduled multiple reaction monitoring (MRM) Algorithm. The acquisition methods consisted of 156 MRM transitions for the NPS panel, 71 MRM transitions for the DOA panel and 3 MRM transitions for the EtG experiment, respectively.

Results: The calibration curves resulting from the NPS panel demonstrated excellent linearity for all the analytes included in the panel with R^2 values >0.98. The robustness of the NPS screening method was further investigated by analyzing 10 authentic hair case samples in which two synthetic cannabinoids JWH-122 and AM-2201 were detected in an authentic case sample at concentrations of 111.8 and 11.72 pg/mg, respectively. The calibration curves resulting from the DOA panel demonstrated excellent correlation of the generated regression curves with R^2 values >0.98 for all the analytes. Ketamine, oxycodone, tramadol and zolpidem were detected in a case sample at concentrations of 0.39, 1602.5, 248.4 and 0.04 ng/mg, respectively. Lastly, four calibration levels ranging from 20 to 300 pg/mg were used to generate the calibration curves for the EtG panel. The regression curves showcase excellent correlation of the generated regression curves with R^2 values of 0.99902 and 0.99917 for each of the EtG transitions. EtG was detected in three case samples at concentration of 7.61, 10.66 and 1.61 pg/mg, respectively.

Conclusion/Discussion: A comprehensive workflow combining the sensitivity of the SCIEX QTRAP 6500+ LC-MS/MS System with a simple extraction procedure enabled detection of a wide variety of drugs and drug metabolites in hair samples. The developed workflow was shown to enable accurate identification and sensitive quantification of a wide range of chemically-diverse analytes included in three different panels. A number of analytes from each panel were accurately detected in authentic hair samples, demonstrating the broad applicability of the sample preparation procedure for the detection of the analytes included in the panels.

POSTER

P-034: Unusual Attempted Drug-Facilitated Sexual Assault Involving MDMA

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Background/Introduction: MDMA is a popular recreational drug. One of the most wanted effects of MDMA is the capacity of this drug to increase empathy and emotional contact. Surprisingly, cases of sexual assault involving MDMA are rare, probably because MDMA does not induce amnesia (Eiden C. et al. A case of drug-facilitated sexual assault involving 3,4-methylene-dioxy-methylamphetamine. *J Psychoactive Drugs* 2013;45:94-7). In this presentation, a case of attempted sexual assault with particularly high concentrations of MDMA in blood and hair will be discussed.

Objectives: A 34-year-old woman was invited by her former husband to have a glass of beer. Alerted by the unusual taste of the drink, she took only one sip and immediately left the apartment. Within a few minutes, she experienced dizziness and a feeling of intense discomfort. She reached her parents' home but very quickly began to experience hallucinations, tachycardia, hyperthermia and excessive sweating. Emergency services were called and the victim was transferred to the hospital. Her condition improved within a few hours and she returned home the next day. On admission to the emergency room, blood and urine samples were taken. Urine immunological screening performed on site showed the presence of MDMA. Therefore, the blood sample was sent to the laboratory for confirmatory analysis. One month later, a 20 cm hair strand, root-to-tip (brown-to-blond) oriented, was collected from the posterior vertex.

Methods: *Blood:* Alcohols were analyzed by HS-GC/FID. Multi-drug blood screening was carried out by liquid chromatography after alkaline extraction, separation on HSS C18 reverse phase column and specific detection by diode array detector and by tandem mass spectrometry (Xevo TQS micro, Waters) with dedicated date rape drugs libraries. A confirmation by HRMS (Waters Xévo G2 QToF) was also performed. New Psychoactive Substances (NPS) were tested by liquid chromatography after alkaline extraction, separation on a C18 reverse phase column and specific detection by tandem mass spectrometry on a Waters Xevo TQD system. GHB was measured by gas chromatography-mass spectrometry. *Hairs:* After segmentation in 3 x 2 cm segments, amphetamines were analyzed by liquid chromatography after alkaline extraction and detection by tandem mass spectrometry on a Waters Xevo TQS micro equipment. The limit of quantification is 1 pg/mg.

Results: Blood: MDMA and MDA were quantified at 189 ng/mL and 13 ng/mL, respectively. GHB determination showed a physiological concentration of 0.85 mg/L. Results were negative for alcohol and other substances.

Hair: MDMA and MDA concentrations are presented in the table below.

Segment	MDMA (pg/mg)	MDA (pg/mg)
0 - 2 cm (period of the events)	936	38
2 - 4 cm	20	Not detected
4 - 6 cm	3	Not detected

Conclusion/Discussion: The blood concentrations of MDMA and MDA are particularly high compared to the cases described in the literature and confirm a recent intake of ecstasy. Hair concentrations of MDMA and MDA are also very high in the segment of interest. This result is most likely due to the fact that the absorbed dose is very high, as shown by the blood concentration. The presence of MDMA in consecutive segments is most likely due to sweat contamination (Kintz P. Hair Analysis in Forensic Toxicology: An Updated Review with a Special Focus on Pitfalls. *Curr Pharm Des* 2017;23:5480-6). From a clinical viewpoint, the massive absorption of MDMA explains the severity of the symptoms presented by the victim. The administration of such quantities of MDMA is surprising in a context of sexual assault. This case highlights the importance of collaboration between emergency physicians and toxicologists in the management of these cases.

P-035: Separation of Four tetrahydrocannabinol isomers: Δ6a/10a, Δ8, Δ9, and Δ10 on a Single Quad LCMS

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Background/Introduction: The cannabis/hemp market continues to grow each year with more states legalizing recreational marijuana, as well as the 2018 Farm Bill removing hemp from the controlled substance list. This bill defines that any cannabis sativa L. strain with a total tetrahydrocannabinol (THC) concentration of 0.3% or less can be considered hemp and not cannabis. Due to this definition there is an even higher demand to differentiate hemp from cannabis by determining the correct concentration of THC. More recently, there are more instances of adulterated hemp samples with THC variants and a need to fully identify and quantify each of these variants. Currently most laboratories are only monitoring and reporting delta-8 and delta-9 THC. This study develops a liquid chromatography mass spectrometry(LCMS) method that separates, identifies, and quantitates delta-8 and delta-9, delta-10 and delta-6a/10a THC.

Objectives: This study focused on developing a chromatographic method for the separation of 4 delta THC isomers in hemp matrix. The final method could be used for both identification and quantitation.

Methods: A Shimadzu LCMS-2020 single quadrupole mass spectrometer coupled with a Nexera 40 series UHPLC system equipped with a PDA was employed for this evaluation. Utilizing a Restek Raptor C18 column (150 mm x 2.1 mm, 2.7 μm) and gradient elution with an overall run time of 8 minutes, four tetrahydrocannabinol isomers, tetrahydrocannabinolic acid, and cannabidiol, were chromatographically separated. Mobile phase A consisted of water with 5mM ammonium formate and 0.1% formic acid and mobile phase B was 50:50 methanol and acetonitrile with 0.1% formic acid for a combined flow rate of 0.7mL/min. The final MS method included both SIM and scan events for each polarity with a Qarray voltage of 55V. The scan events monitor for in source collision induced disassociation (CID). Linear calibration curves were created as neat standards. and were run from 10 ng/uL to 0.01 ng/uL.

The final THC content was calculated using the two different calculations shown below.

Equation 1: (%THCA x 0.877) + %Δ9-THC = potency of THC

Equation 2: (%THCA x 0.877) + %ΔTHC sum of isomers = potency of THC

Results: Separation of delta-6a/10a THC, delta-8 THC, delta-9 THC, and delta-10 THC was achieved in a single chromatographic run. All the THC isomers plus CBD have the same mass and therefore could only be differentiated using retention time. With simultaneous in-source CID additional identification could be completed by comparing the fragmentation patterns produced for each isomer. When comparing the four THC isomer's fragmentation patterns the fragment *m/z* 193 and *m/z* 259 is consistent for all isomers, but *m/z* 247 is only present with delta-8 and delta-9 THC. Whereas fragment *m/z* 299 is only present for delta-6a/10a THC, and fragment *m/z* 217 is only present for delta-10 THC. All calibration curves were run in triplicate and had at least 4 points with a $R^2=0.998$ or better. The average accuracy for each level was between 95% and 108%. Hemp samples were analyzed against the calibration curve to determine the %THC present in each sample.

Conclusion/Discussion: This application demonstrates chromatographic separation of four THC isomers using a single method. The single quadrupole mass spectrometer, LCMS 2020, demonstrated its capability for simultaneous detection and conformation using in source fragmentation of all analytes. Linear calibration curves were acquired for each analyte and hemp samples were analyzed to determine their THC content. The final statistical results show retention time and peak area repeatability, quantitative accuracy and sensitivity.

Figure 1. Representative SIM Chromatogram for 4 THC isomers at 0.1ng/uL
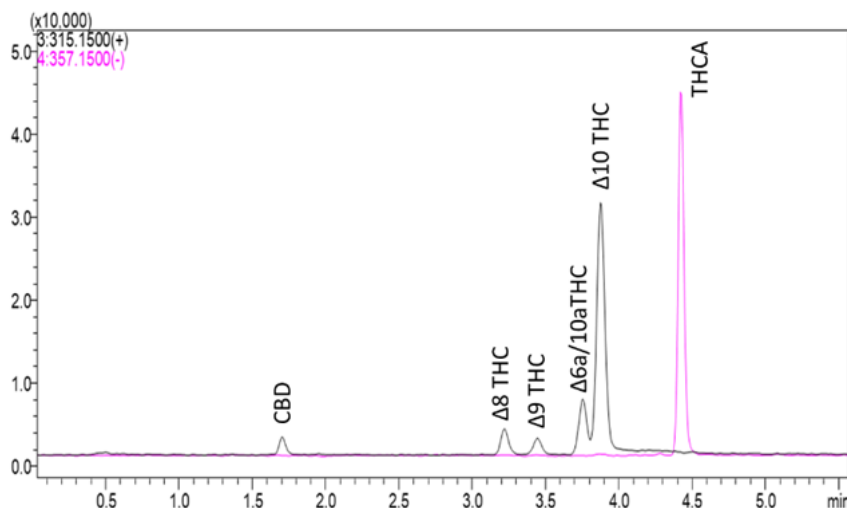
POSTER

P-036: Rapid and automated analysis of drugs of abuse in oral fluid using extraction (XTR) tips

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Background/Introduction: Analysis of oral fluid generally requires some type of sample preparation to concentrate the analyte and reduce matrix effects for sensitive and reproducible analyses. The use of dispersive pipette extraction (XTR) tips has been previously shown to offer advantages over other solid-phase extraction (SPE) products in terms of speed and feasibility with automation. Most SPE methods for analyzing drugs of abuse in oral fluid incorporate a strong acid in order to achieve high recoveries of basic drugs using strong cation exchange sorbent. However, there have been reports that this acid may lead to conversion of cannabidiol (CBD) to tetrahydrocannabinol (THC). In this research study, we used mixed mode XTR tips without the addition of a strong acid in order to avoid possible conversion of CBD to THC. This study focuses on analyzing 41 drugs of abuse commonly found in driving-under-the-influence (DUI) cases. The analysis uses a single extraction method and high performance liquid chromatography with tandem mass spectrometry (LC/MS/MS). A separate LC/MS/MS method is also presented for differentiation of delta-8 and delta-9 THC (for positive THC cases).

Objectives: The objective of this study is to develop a rapid and automated method for analyzing comprehensive drugs of abuse in oral fluid for driving-under-the-influence casework. The use of mixed-mode sorbent is evaluated to provide reproducible and high recoveries of acidic, neutral and basic drugs commonly found in DUI investigations. A separate LC method from the comprehensive method is used in order to differentiate delta-8 THC from delta-9 THC.

Methods: Synthetic negative saliva (Immunalysis Corp.) buffer solutions (500 μL) were fortified with 50 μL of a comprehensive drug standard (45 drugs) that includes opiates, opioids, benzodiazepines, barbiturates, THC (delta-8 and delta-9, as well as CBD), antidepressants, amphetamines, and cocaine. Concentrations ranged, depending on the analytes, as low as 0.1 ng/mL to as high as 500 ng/mL. A Hamilton Nimbus-96 was used to perform all extractions of oral fluid. The extraction involved the steps of conditioning with 800 μL 50% methanol in water, aspirating and dispensing the solution 5 times, washing with 800 μL of water, and elution with 500 μL of 48/48/4% acetonitrile/methanol/ammonium hydroxide. The solutions were subsequently evaporated and reconstituted in 125 μL 10% methanol in water. All analyses were performed using a SCIEX 6500+ MS system coupled to an Agilent 1260 LC system using mobile phases of pH 3.6 ammonium formate in water and methanol. For comprehensive analysis, a Phenomenex Kinetex 2.6um biphenyl column (50 x 3.0 mm) was used. For separating CBD, delta-8 and delta-9 THC, a Restek Raptor FluoroPhenyl (2.7 μm, 100 x 2.1 mm) was used. The extraction studies included the analysis of 24 samples extracted weekly for 2 months, for a total of 192 samples.

Results: The extractions using the XTR tips on the Hamilton Nimbus-96 took less than 10 minutes to complete (for 96 samples simultaneously). Almost all of the drugs had recoveries greater than 80%. Drugs fortified at suggested cutoffs (from the literature) were readily detected at less than 8% C.V. The comprehensive LC/MS/MS method was used to detect THC and CBD, but a separate LC/MS/MS method was used to detect delta-8 and delta-9 THC at levels as low as 0.5 ng/mL.

Conclusion/Discussion: This study demonstrates a rapid, efficient and automated method for analyzing comprehensive drugs of abuse in oral fluid using mixed-mode XTR tips.

P-037: Drugs of Abuse in Urine and Plasma Extracted with Microelution SPE Technology and Analyzed via LC-MS/MS

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Background/Introduction: Routine analysis of samples in clinical and forensic settings demands quick and efficient extraction procedures. Smaller sorbent amounts utilized by Solid Phase Extraction (SPE) products allow scaling-down of starting sample size and minimize the total wash and elution solvent volumes. The use of 2 mg or less of sorbent particles embedded in a disc membrane allows for sample enrichment and high throughput processing. As compared to loose sorbent, disk eliminates channeling effects and reduces dead volume. Removal of the evaporation step from the procedure also decreases overall turn-around time. This poster outlines methods for extracting large drugs of abuse panel from urine and plasma using reverse-phase and mixed-mode sorbent chemistries in microelution format.

Objectives: To develop and validate extraction procedures for drugs in urine and plasma utilizing reverse phase and mixed-mode microelution plate chemistries.

Methods: Drug-free human urine and defibrinated plasma were obtained from UTAK laboratories. The samples were extracted on UCT's Micro-Prep® HLB and MMCX microelution plates. Calibration standards were prepared in a range from 1 ng/mL to 200 ng/mL and quality control samples were spiked at 5 ng/mL and 50 ng/mL. Sample processing on HLB: 300 mcL sample was diluted 1:1 with Carbonate-Bicarbonate buffer (pH 10.0, 0.1 M) followed by the addition of an assay appropriate internal standard. The diluted sample (400 mcL total volume) was then loaded onto the HLB plate. Matrix interferences were washed with 100 mcL 5% methanol and the analytes of interest were eluted using 50 mcL 2% formic acid in methanol. Sample processing on MMCX: 300 mcL sample was diluted 1:1 with Phosphate buffer (pH 6.0, 0.1 M) followed by addition of internal standards. 400 mcL of the total diluted sample was then transferred onto the MMCX plate. Wells were subjected to a series of wash steps in the sequence of 100 mcL 0.1 M acetic acid and 100 mcL 40% methanol. 50 mcL 2% ammonium hydroxide in methanol was used as the elution solvent which was then neutralized with 50 mcL 2% Formic acid in DI water. The collection plates were loaded onto the Shimadzu Nexera X2 SIL-30AC autosampler for analysis using a Shimadzu LCMS-8050 Triple Quadrupole Mass Spectrometer. Water and methanol consisting of 5 mM ammonium formate were used as mobile phase (0.4 mL/min flow rate over a 13 minute run time). UCT's Selectra® PFPP UHPLC column was employed to carry out analytes separation.

Results: HLB microelution plate utilized to extract urine and plasma quality control samples yielded excellent recoveries for a majority of the analytes in the panel. From a total of 47 drugs, >80% recoveries were achieved for 37 drugs fortified at 5 ng/mL and for 43 drugs spiked at 50 ng/mL. Corresponding RSD values were <10% at both concentration levels. From a total of 50 drugs extracted on MMCX microelution plate, 45 and 48 drugs showed >80% recoveries at 5 ng/mL and 50 ng/mL respectively. The RSD values for both concentrations were <20%.

Conclusion/Discussion: Excellent recoveries and relative standard deviation (RSD) values confirm both the microelution extraction methods to be efficient. In addition to using minimal wash and elution solvent volumes, the elimination of the drying step reduced the overall processing time to approximately less than 30 to 40 minutes. The potential for automation and the option to load the collection plate directly on to the autosampler make this extraction technique very convenient for high throughput forensic and clinical labs.

POSTER

P-038: Sevoflurane as an Interferent in HS/GC Analysis of Volatiles

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Background/Introduction: Sevoflurane ($C_4H_3F_7O$) is a sweet-smelling, non-flammable, fluorinated methyl isopropyl ether used as an inhalational general anesthetic. Introduced into the clinical market in 1990, it has become widely used throughout the world. Sevoflurane has many clinical advantages including low blood-gas solubility, rapid induction of anesthesia and quick emergence and recovery at the end of anesthesia. It is non-irritating to the airway, pleasant smelling, nonexplosive, nonflammable, and relatively inert. It has a molecular weight of 200 g/mol and a boiling point of 58.5 °C. Sevoflurane and other fluranes have been used recreationally and in suicides. It may also be seen in traffic related cases and other death investigations.

Objectives: The goal of this study was to evaluate sevoflurane as a potential interferent in four different HS/GC instruments used at the Alabama Department of Forensic Sciences (ADFS) for the analysis of ethanol and other volatiles.

Methods: Ethanol and mixed volatile standards were obtained from Lipomed and Cerilliant, respectively. Sevoflurane obtained from Sigma-Aldrich was evaluated under validated methods using the following instruments: Agilent Headspace G1888-Gas Chromatograph 7890A/Mass Spectrometer 5975C with Agilent DB-624 UI single capillary column (Inst. 1), Hewlett-Packard Gas Chromatograph HP6890/Teledyne Tekmar Headspace Analyzer (HT3) with Alltech 5% Carbowax 20M Carbopack packed column (Inst. 2), and Agilent Gas Chromatograph 6890N/Teledyne Tekmar Headspace Analyzer (HT3) with Restek Rtx-BAC1 and -BAC2 dual-capillary columns (Inst. 3), Agilent Gas Chromatograph 8890/Agilent Headspace 7697A with Restek Rtx-BAC1 and −BAC2 dual-capillary columns (Inst. 4). A solution of sevoflurane at approximately 67 mg/L was aliquotted with n-propanol/sodium fluoride mix as internal standard and injected on each instrument to determine the retention time of sevoflurane. An ethanol control (0.15 g/100mL) was spiked with approximately 67 mg/L and a mixed volatile (acetone, ethanol, isopropanol, and methanol) control (500 mg/L) was spiked with varying concentrations of sevoflurane and injected on each instrument to evaluate potential interference and co-elution. Biologically relevant concentrations of sevoflurane were analyzed.

Results: The retention times for sevoflurane and ethanol on instruments 1, 2, 3, and 4 are listed in Table 1 below. The major ions for sevoflurane and ethanol were 131, 69, 181 and 45, 31, 46 m/z, respectively, by HS/GC/MS. HS/GC instruments 1 and 2 fully resolved sevoflurane from ethanol and related volatiles and displayed no interference. These instruments are used primarily for screening (qualitative purposes) at ADFS. However, sevoflurane had a similar retention time to ethanol on instruments 3 and 4. On BAC 1 and BAC 2, sevoflurane co-eluted with ethanol. Instruments 3 and 4 are primarily used for quantitative purposes at ADFS. Authentic sevoflurane cases were isolated for illustration purposes.

Table 1.

	Inst. 1	Inst. 2	Inst. 3		Inst. 4	
	DB-624	5% Carbowax 20M	Rtx-BAC1	Rtx-BAC2	Rtx-BAC1	Rtx-BAC2
Sevoflurane	2.55	5.33	1.28	1.16	1.26	1.13
Ethanol	**2.65**	**1.79**	**1.24**	**1.16**	**1.22**	**1.13**

Conclusion/Discussion: It is important to perform thorough interference studies for all analytical methods. Laboratories should evaluate sevoflurane as a potential interferent during volatiles analysis for existing methods and/or future validated methods. In this study, sevoflurane interferes with our HS/GC/FID quantitative method, but would be detected during our initial HS/GC/MS qualitative screen. The use of a dual-column configuration and multiple detector technologies (i.e. FID and MS), helps to identify and detect interferents. A workflow involving a screen and confirmation conducted in separate analyses and the addition of HS/GC/MS analysis provides enhanced specificity and the ability to monitor other volatile substances such as sevoflurane.

POSTER

P-039: Multi-Target Screening and Quantitative Method Validation of 24 Drugs in Synthetic Urine Using Automated Sample Preparation Coupled Directly to LC-MS/MS

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Background/Introduction: In forensic toxicology, analysis of drugs in biological samples is conducted to identify drug-facilitated crimes, drug abuse, or whether someone was driving under the influence (DUI(D)). Determining what analytes are present and the concentration of those compounds in a variety of matrices (e.g., blood, urine, or oral fluid) can be complex. Sample preparations in most forensic laboratories commonly use solid-phase extraction (SPE) or liquid-liquid extraction (LLE), which are not only time-consuming, but also introduce human errors during the multi-step protocols.

Objectives: We describe an automated alternative platform using a sample preparation module (CLAM-2030™) connected to a Liquid Chromatograph-tandem Mass Spectrometer (LC-MS/MS) for multi-target screening and quantitation of 24 commonly abused drugs in urine.

Methods: Mixtures of 24 drugs were spiked at varying concentrations using certified reference standards. The automated sample preparation module is fully integrated, conducting dilution, protein precipitation, stirring, and vacuum filtration. It can process several samples simultaneously, which increases throughput. Sample preparation was performed entirely inside the CLAM-2030 module, using the following pre-programmed steps: wetting polytetrafluoroethylene filtering vials with solvent (e.g. methanol, acetonitrile, etc.), sample dispensing, reagent dispensing for internal standard (IS) addition and protein precipitation, sample shaking inside the module for 30 seconds at 2000 rpm, vacuum filtration for 90 seconds into collection vial, and finally transferring the collection vial directly into the autosampler of the LCMS-8050 system. Compared to manual sample preparation, the time was reduced from over 2 hours to 5 minutes. A ready-to-use method package Forensic Toxicology Database, which includes methods, multiple reaction monitoring (MRM) transitions and libraries of over 2000 analytes, was used to set up the LC-MS/MS screening method for the analytes. Retention times of the analytes were updated using certified reference standards and MRM optimization of the ISs was carried out. Two or more MRM events were used to identify compounds. The whole workflow was run automatically for an entire batch, including calibrators, matrix blanks, spiked biological samples and controls. Following the method LC conditions, the chromatographic separation of the drugs was achieved by a gradient elution in 17 minutes. Additionally, screening results were compared to the forensic toxicology database library for identification using similarity scores.

Results: Validation of this quantitative method was completed following ANSI/ASB Standard 036. Limit of quantitation (LOQ) were as low as 1 ng/mL for all 24 analytes. Linear calibration curves in the range of 1-100 ng/mL were established for the 24 analytes with R^2 ≥0.995. Bias and precision were evaluated at three concentrations and none exceeded the 20% acceptance range. No significant carry-over was observed, even after the highest calibrator. No interferences from matrix and internal standards were observed.

Conclusion/Discussion: A fully integrated and automated platform consisting of CLAM-2030 and LC-MS/MS was used in establishing a multi-target screening method with quantitation for 24 drugs in synthetic urine. The automated sample preparation approach offers a reproducible solution, which helps eliminate human errors during manual preparations and increase laboratory safety. By using the Forensic Toxicology Database, tedious method development work was avoided, as only retention time adjustments and MRM optimization for some ISs were needed. This work demonstrates that the system, which couples automated sample preparation and LC-MS/MS, can be effective in high-throughput screening of large numbers of targets in biological samples in forensic toxicological research and investigation.

POSTER

P-040: Evaluation of Sample Preparation Approaches for the Extraction of Amphetamine, Methamphetamine, MDMA and metabolites from Urine prior to GC/MS Analysis

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Background/Introduction: Amphetamine, methamphetamine and ecstasy continue to be widely abused in many parts of the world with urine analysis continuing to be the most popular approach to determining drug intake.

Objectives: The aim of this poster is to investigate various sample preparation approaches for the extraction of amphetamines from urine. Optimized methods using solid phase extraction (SPE), supported liquid extraction (SLE) and a novel approach of dual mode extraction (DME) were compared for recovery, reproducibility, sensitivity, calibration, procedural simplicity, solvent consumption, associated assay time and overall cost.

Methods: Negative urine was provided by healthy human donors and spiked with an analyte mix of amphetamine, methamphetamine, MDA, MDMA and MDEA. Amphetamine–D_5 was utilised as the internal standard and spiked into samples prior to pre-treatment. Analyte extraction was investigated using various sample preparation approaches: silica and polymer-based SPE, supported liquid extraction (SLE) and dual mode extraction (DME). Optimized procedures were evaluated for overall performance. Extracts were evaporated at ambient temperature and concentrated if applicable, followed by in-vial derivatization with ethyl acetate (50 μL) and pentafluoropropionic anhydride (PFPA) (50 μL). The samples were capped and heated at 50 °C for 15 minutes then cooled. The samples were once again evaporated at ambient temperature and reconstituted in ethyl acetate for analysis. GC/MS analysis was performed using an Agilent 7890 GC and a 5975 MSD, following sample injection of 2 μL in splitless mode. Chromatography was performed on a Restek Rxi® -5ms capillary column; 30 m x 0.25 mm ID x 0.25 μm using 1.2 mL/min helium. Positive ions were acquired using electron ionization operated in SIM mode.

Results: Silica-based SPE has historically been used for urinary amphetamine analysis and was the benchmark for this study. Optimized silica-based SPE returned clean extracts and typical recoveries in excess of 95%. The polymer-based SPE approach allowed recoveries of 87-102% with RSDs below 5% using full and simplified load-wash-elute extraction protocols. The supported liquid extraction approach allowed recoveries of 97-108% with RSD values below 10%. Dual mode extraction returned recoveries slightly lower than the other techniques, generally up to 80% with corresponding RSDs below 10%. Calibration curves were constructed from 10-1000 ng/mL with Amphetamine-D5 used as internal standard spiked at 100 ng/mL. Excellent linearity below the SAMHSA/ EWDTS confirmation cut offs of 250/200 ng/mL respectively for workplace testing applications and coefficients of determination (r^2) greater than 0.99 were returned from all tested procedures. Although excellent sensitivity and overall performance silica based SPE suffers from some drawbacks not associated with modern techniques: they require extensive phase conditioning, often methanol followed by at least one aqueous buffer for pH control of the sorbent. These steps add time, solvent usage and complexity to each assay. Modern polymer-based SPE such as EVOLUTE EXPRESS contain water wettable components allowing the elimination of phase pre-conditioning. The resulting load-wash-elute protocol provides cost saving in terms of solvent use and disposal. The more retentive nature and higher capacity of polymer-based SPE also allow column size reduction which consequently led to reduced wash and elution volumes. Likewise the use of supported liquid extraction allows a streamlined processing load-wait-elute protocol to be used. Simple processing, no waste but the use of water immiscible organic solvents mean evaporation is almost always required. Finally techniques such as dual mode extraction employ a simple acetonitrile precipitation followed by flow through processing. The media scavenged urinary components resulting in an acetonitrile rich elution.

Conclusion/Discussion: This poster demonstrates multiple approaches for the extraction and cleanup of amphetamines from urine. Various sample preparation approaches exhibit the required method performance in terms of recovery, extract cleanliness and sensitivity. However, individual laboratory perspectives in terms of cost, assay time, solvent and reagent use may dictate final choice of technique.

P-041: Modification and Validation of EMIT Oxycodone Urine Screening Kits for use with Serum

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Introduction: Enzyme Multiplied Immunoassay Technique (EMIT) is a common technique used for drugs of abuse and therapeutic drug testing, such as oxycodone. EMIT kits are commonly designed and used for the screening, semi-quantitation, or quantitation of drugs in urine and serum. EMIT methods can be modified for use with alternate matrices to provide rapid analysis with limited required sample preparation by adjusting sample and reagent volumes. Oxycodone is a semi-synthetic opioid used in the treatment of acute and chronic pain. Metabolites of oxycodone include noroxycodone and oxymorphone, which both have analgesic effects. EMIT could provide rapid semi-quantitation of oxycodone in serum without the need for tedious and time-consuming extractions. Presented is the evaluation for limit of quantitation, inter and intra-day bias and precision, and specificity of three different EMIT oxycodone kits using in-house oxycodone fortified human serum calibrators and controls. Optimized EMIT kits were evaluated with genuine serum oxycodone samples. These samples were collected as part of an ongoing human study at the Virginia Commonwealth University Institute for Drug and Alcohol in accordance with IRB #HM20008062. Patients received three oral doses of oxycodone immediate release (OxyIR) 45 minutes apart. Blood was collected 15 minutes prior to the first dose and 30, 45, 150, and 450 minutes after the last dose. Results of the optimized kits were compared to concentrations determined using a high-performance liquid chromatography tandem mass-spectrometry (HPLC-MS/MS) method.

Objective: To evaluate the potential use of three different EMIT Oxycodone testing kits to semi-quantitate oxycodone in serum after optimization.

Methods: Three urine EMIT kits were evaluated, the Lin Zhi (LZI), Lin Zhi research (LZI-R), and Syva Emit II Plus Oxycodone Assay. The analysis was performed using an Abbott Architect Plus c4000. The EMIT kits were optimized for reagent and sample volumes. Evolution of the optimized methods was performed using six-point calibration curves, ranging from 10 to 150 ng/mL, and quality control specimens prepared at 10, 30, 75, and 125 ng/mL in serum over three days. Genuine serum oxycodone samples (n=27) were then evaluated by the three urine EMIT kits. Results from the EMIT kits were compared to HPLC-MS/MS results.

Results: The optimized volume for reagent 1, reagent 2, and sample volumes were determined to be: LZI (140, 65 and 15 µL); LZI-R (140, 65 and 12.5 µL); and the Syva Kit (140, 90 and 9.8 µL). The EMIT methods exhibited overall good linearity, accuracy, and precision. The lower limit of quantitation quality (LLOQ) control sample exhibited the highest intraday and interday bias and precision values for all kits. Intraday bias of the LLOQ for LZI, LZI-R, and Syva were 17%, 24%, and 11% respectively. For all kits, the intraday precision of the LLOQ was <13 %, other quality control samples were <6%. Genuine sample concentrations determined by HPLC-MS/MS compared to EMIT kits (% difference range): LZI: -8 to 150%, LZI-R: -1 to 122%, and SYV: 16 to 262%. The elevated concentrations were likely the result of cross reactivity of metabolites to the EMIT assay as the disparity increases at later time points at which the concentration of metabolites would be increasing, and the oxycodone would be decreasing.

Conclusion: Lin Zhi, Lin Zhi research, and Syva EMIT oxycodone kits were effective in detecting oxycodone in serum. While each EMIT assay reported elevated concentrations of oxycodone compared to the HPLC-MS/MS method, the methods were effective for semi-quantitative testing.

POSTER

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P-042: Efficient Removal of Beta-glucuronidase Enzyme for Quantitative Analysis of Therapeutic Drugs and Drugs of Abuse in Urine by LC-MS/MS

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Background/Introduction: It is widely accepted in urine drug testing (UDT) that enzymatic hydrolysis is the preferred practice to increase target analyte detection limits, minimize cost, and improve clinical utility of the assay. The addition of beta-glucuronidase for hydrolysis of urine samples has been vastly adapted in labs across the globe. Since cutting lab expenses continues to be a focal point in many of today's UDT labs, dilute-and-shoot sample analysis has become more prevalent. However, due to its potential impact on LC-MS/MS robustness, labs must balance the sample dilution factor, analyte sensitivity, and avoid injecting added proteins from the beta-glucuronidase into the system. Large dilutions or protein precipitation with centrifugation are standard protocols in clinical and forensic laboratories alike. Achieving the necessary cutoff levels robustly can be challenging, especially without the luxury of high-end LC-MS/MS instrumentation. In this presentation, we demonstrate an improved and automated product and process that will rapidly remove the enzyme and other proteins from a urine sample, post hydrolysis, via filtration through a proprietary membrane. The product minimizes the required sample dilution factor that is otherwise necessary, improves the limits of detection for all test method analytes, and does not require protein precipitation with off-line centrifugation. This novel automated product removes the protein content from the sample, providing beta-glucuronidase free injection on the LC-MS/MS.

Objectives: The primary goal was to develop a completely automated product to rapidly remove beta-glucuronidase from hydrolyzed urine samples without requiring a large dilution with organic solvent or hindering recovery of analytes of interest. As a result, the process provides clean samples with minimal dilution requirements and increased sensitivity.

Methods: Kura Biotech's B-OneTM All-In-One hydrolysis solution was chosen for enzymatic hydrolysis. A 35 µL aliquot of B-One enzyme was added to 35 µL of urine sample. Internal standard (20 µL) was added to the sample solution following an additional 35 µL of water. The solution was hydrolyzed at room temperature for 15 minutes. Post-hydrolysis, the 125 µL solution was passed through the DPX membrane device that holds a proprietary membrane designed for enzyme removal. After passing the solution through the device, the filtrate is ready for injection. At only 15% methanol, the solution does not require any additional dilution for LC-MS/MS analysis. The recovery and precision of a variety of therapeutic and abused drugs were monitored. Protein content of beta-glucuronidase was also monitored via gel electrophoresis.

Results: The amount of beta-glucuronidase in the unfiltered sample and the filtered sample was observed via gel electrophoresis as shown in Figure 1. The pictured gel demonstrates greater than 80% removal of the beta-glucuronidase enzyme. The recovery of the analytes monitored was found to meet acceptance criteria according to SAMSHA recommendations. Coupled with the minimal required dilution factor compared to traditional dilute-and-shoot protocols, this method provides the sensitivity required for clinical, forensic, and employment drug testing labs.
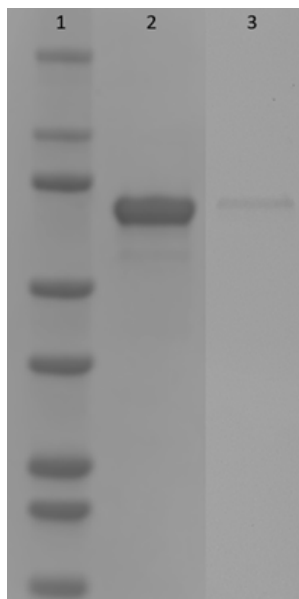
Figure 1. Lane 1 is the protein marker. Lane 2 is the unfiltered sample. Lane 3 is the filtered sample.

Conclusion/Discussion: This study demonstrates a rapid and effective protein removal product and process that enables high recoveries of the target analytes for comprehensive urine panels and is highly effective at removing proteins from hydrolyzed urine samples. The product is cost-effective, and the process is completely automated using Hamilton Robotics.

POSTER

P-043: Method Validation for the Identification of Fentanyl, Fentanyl Analogs, and Other Synthetic Opioids in Blood, Liver, and Urine by LC-MS/MS

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Background/Introduction: Fentanyl and the proliferation of its analogs along with other synthetic opioids continue to contribute to the ongoing opioid crisis in the United States. Many compounds are scheduled or included under emergency scheduling for controlled substances, but still continue to be illicitly manufactured and abused. Since these compounds have varying degrees of potency, it is important to develop sensitive methods to characterize these compounds in a variety of matrices. Analyzing these compounds in different types of casework such as driving under the influence of drugs (DUID), drug-facilitated crime (DFC), and postmortem cases can help inform public health and law enforcement officials of the prevalence of use of these compounds in communities.

Objectives: To validate a method for the analysis of fentanyl, its analogs, and other synthetic opioids in blood, liver, and urine using solid phase extraction (SPE) and liquid chromatography tandem mass spectrometry (LC-MS/MS) for the purpose of evaluating these compounds in casework in Harris County.

Methods: A solid phase extraction was performed to isolate 4-fluoroisobutyryl fentanyl (4-FIBF), acetyl fentanyl, acryl fentanyl, alfentanil, butyryl fentanyl, carfentanil, cyclopropyl fentanyl, fentanyl, furanyl fentanyl, methoxyacetyl fentanyl, norcarfentanil, norfentanyl, sufentanil, U-47700, U-48800, U-49900, and valeryl fentanyl from 0.2 mL of blood, liver, or urine. An Agilent 6460 Triple Quadrupole Mass Spectrometer operated in positive mode electrospray ionization (ESI) was used for the detection of the previously listed analytes. Chromatographic separation was achieved with gradient elution using an Agilent Poroshell 120 Phenyl Hexyl guard and column. Mobile phases consisted of 0.1% formic acid in water and 0.1% formic acid in acetonitrile. This assay was validated in accordance with ANSI/ANAB Standard 036, Standard Practice for Method Validation in Forensic Toxicology, and conforms to criteria outlined by the American Board of Forensic Toxicology. Parameters evaluated were precision, bias, calibration model, carryover, interferences, ion suppression/enhancement, limit of detection, limit of quantitation, dilution integrity, and processed sample stability.

Results: A total of 17 analytes were assessed at concentrations ranging from 0.05-100 ng/mL in blood, liver, and urine. A weighted (1/X) quadratic calibration model of 0.5-100 ng/mL was selected for quantitative analytes in blood, which was also used for the quantitation of liver controls. Accurate and precise quantitative analysis was achievable for eight compounds in blood, and five compounds in liver. Qualitatively identified analytes in blood and liver were alfentanil, butyryl fentanyl, norcarfentanil, norfentanyl, sufentanil, U-47700, U-48800, U-49900, and valeryl fentanyl using a single-point curve. All analytes were qualitatively assessed in urine. Limits of detection ranged from 0.05-0.5 ng/mL in all three matrices. Ion suppression results were variable between matrices but demonstrated no impact on other critical validation parameters. There were no interferences from other common analytes, internal standards, or matrix, with the exception of ketamine which shared a qualitative ion transition with norfentanyl-D5. Dilution integrity was assessed up to 10X and was acceptable for all quantitative analytes. All analytes were stable for the full 7-day processed sample stability window that was evaluated and carryover was acceptable.

Conclusion/Discussion: Prior to the development and validation of this method, fentanyl was the only analyte from this panel that was quantitatively evaluated by LC-MS/MS at the Harris County Institute of Forensic Sciences. This method improves and expands upon the previous method scope by lowering the detection limits for fentanyl from 1 ng/mL in blood to 0.05 ng/mL, and by adding compounds that have not been previously characterized in our laboratory. Evaluating the presence and concentrations of these other synthetic opioids and fentanyl-related drugs in casework will provide information about the prevalence of these compounds in Harris County and the role they may play in various types of casework.

POSTER

P-044: Rapid Screening of 65 Common Drugs and Drug Metabolites in Urine and Blood Using High-Resolution Mass Spectrometry

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Background/Introduction: Drug abuse has become one of the most serious social issues worldwide as drugs continue to pose a threat to social stability and economic development. As the surge of new designer drugs continues to pose public health and safety problems, drug testing remains one of the most effective measures for global drug control. The ability to swiftly detect them and their metabolites in the blood and urine of drug users is paramount for law enforcement and testing departments who require comprehensive drug screening approaches with high-level sensitivity and specificity.

Objectives: The objective of this study is to develop a comprehensive acquisition method enabling rapid detection, identification, and quantitation of 65 drugs and drug metabolites from urine and blood samples using high-resolution mass spectrometry. The method combines a simple protein precipitation procedure and a unique SWATH Acquisition with MRM[HR] workflow for high sensitivity detection of drugs and drug metabolites in biological samples.

Methods: A total of 65 drugs and drug metabolites were selected for this panel. Drugs and drug metabolites were extracted from blood and urine samples by using a protein precipitation procedure. Control blood and urine samples spiked with the 65 drugs and drug metabolites were prepared at various concentrations ranging from 5 to 50 ng/mL. These standard mixtures were extracted using the aforementioned procedure and injected to build a data processing method. Analytes were chromatographically separated at 40˚C using a Phenomenex Kinetex C18 column (50 × 3 mm, 2.6μm, 00B-4462-Y0). Mobile phases used consisted of ammonium acetate, acetonitrile, and appropriate additives. The flow rate was 0.3 mL/min. The injection volume was 5 µL and the total LC runtime was 8.5 minutes. MS and MS/MS data were collected using the SWATH Acquisition combined with MRM[HR] workflow on the SCIEX X500R QTOF System with SCIEX OS Software 1.5.

Results: The quantitative performance of the assay was investigated by injecting a series of samples extracted from blood and urine samples at three concentration levels (5, 10 and 50 ng/mL). Calibration curves showed a high level of consistency and precision across the calibration series. In addition, excellent linearity across the calibration range was observed with R^2 values above 0.99 for all the drugs and drug metabolites in this panel. The recovery was calculated for each of the three concentration levels (5, 10 and 50 ng/mL). The protein precipitation procedure used in this experiment demonstrated recoveries between 77.0% and 118.8% for all the drugs and drug metabolites, which met the requirements for large scale detection of drugs from biological samples. The overall robustness of the workflow was further investigated by analyzing five forensic biological case samples. Of the five samples, one blood sample (S1) and two urine samples (S2 and S3) tested positive for several drugs and drug metabolites including 6-monoacetylmorphine, morphine, methamphetamine, and codeine. SWATH Acquisition enabled generation of high-quality MS/MS spectra which allowed positive identification of the drugs through spectral library matching.

Conclusion/Discussion: A comprehensive workflow for the detection of 65 drugs and drug metabolites in blood and urine samples was successfully developed using the SCIEX X500R QTOF System. The results from the analysis of forensic biological case samples show that the combined SWATH Acquisition with MRM[HR] workflow enabled sensitive detection and accurate identification of drugs and drug metabolites. The information that can be inferred from the results offer a valuable insight on drug intake.

POSTER

P-045: Intracranial self-stimulation in rats as a model of abuse potential and a predictive tool for threat assessment of emerging drugs.

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Background/Introduction: Novel psychoactive substances (NPS) often emerge when previous NPS are detected and controlled. The consequences of this continuous evolution are a lack of bioanalytical methods for their analysis and a scarcity of pharmacological data regarding drug effects. Pharmacological evaluation of drugs typically begins with chemical analysis and in silico evaluation, followed by in vitro testing for properties such as receptor affinity, and finally by characterization through in vivo assays. This process is time consuming and incompatible with the rapid responses needed for forensic, regulatory, and public health services. We propose the use of intracranial self-stimulation (ICSS), a well-established in vivo bioassay for abuse liability testing in rats, as a preliminary screen for threat assessment and pharmacological evaluation of emerging drugs. ICSS is an operant behavioral procedure in which rats are trained to press a lever to receive pulses of electrical brain stimulation that activate the mesolimbic dopamine system, a key component of the brain's reward system. The "dose" of stimulation is manipulated by changing the stimulation frequency, and increasing frequencies of stimulation maintain increasing rates of ICSS responding. This behavior is sensitive to perturbations induced by administered drugs and can be quantified. Facilitation of low ICSS rates maintained by low brain-stimulation frequencies is associated with increased abuse potential. Here, we compared the potencies, maximal effects, and time courses of several dopaminergic drugs on ICSS and present an evolving strategy to triage emerging drugs of abuse.

Objectives: The objective of this research is to develop and characterize a threat-assessment protocol for emerging drugs using ICSS for abuse liability testing in rats.

Methods: Electrodes targeting the medial forebrain bundle were implanted in rats using a stereotaxic surgical procedure and permanently affixed to the skull. Rats were trained to depress a lever to receive electrical brain stimulation at a fixed amplitude and variable frequency in components consisting of ten one-minute frequency trials. Following acquisition of lever-pressing behavior, groups of rats (typically n=6) were administered a test drug via intraperitoneal injection and their responses were monitored for dose- and time-dependent alterations in rate. Test drugs included R(+)-methcathinone (0.10 – 3.2 mg/kg), S(-)-methcathinone (0.10 – 1.0 mg/kg), α-PHP (0.32 – 3.2 mg/kg), cocaine (1.0 – 10 mg/kg), and two cocaine analogs with slower onsets of action [WIN-35428 (0.10 – 0.32 mg/kg), and RTI-31 (0.032 – 0.10 mg/kg)].

Results: Under baseline conditions, electrical brain stimulation maintained a frequency-dependent increase in reinforcement rates. Each of the six drugs facilitated ICSS in a dose- and time-dependent manner. R(+)- and S(-)-methcathinone differed in their potency to facilitate ICSS responding by a factor of 2.4, and demonstrated a rapid onset of effects. α-PHP and cocaine both exhibited robust and rapid increases of rates of ICSS. The cocaine analogs WIN-35428 and RTI-31 also facilitated ICSS with higher potencies, similar maximal effects, and delayed onsets compared to cocaine.

Conclusion/Discussion: ICSS is especially sensitive to the abuse-related effects of drugs that function as substrates or inhibitors of the dopamine transporter, and each of the compounds tested here exhibited robust ICSS facilitation indicative of high abuse potential. Studies with enantiomers of methcathinone highlighted ICSS sensitivity to potency differences between drugs and drug isomers. ICSS can also establish drug time course, which is relevant because faster onset correlates with higher abuse potential for mechanistically similar drugs. All six compounds tested here would be triaged into a high threat priority 1 category based upon their magnitudes of ICSS facilitation, but the additional consideration of delayed onset and would rank WIN-35428 and RTI-31 as lower threats than the other drugs. These data demonstrate the usefulness of ICSS as a rapid tool to assess NPS as they emerge for abuse liability and potential community threat.

POSTER

P-046: Do you have a parent present? Desalkylflurazepam detection in the absence of a parent compound

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Background/Introduction: Historically in Orange County, California, desalkylflurazepam has been detected with parent flurazepam or other flurazepam metabolites. Flurazepam has a half-life of 1-3 hours, while its metabolites persist much longer in the body, so cases where only desalkylflurazepam and other flurazepam metabolites are detected are common. However, beginning August of 2020 desalkylflurazepam appeared on its own in an increased number of cases. Confirming this unexpected finding, the seized drug section of the laboratory identified two cases of desalkylflurazepam in counterfeit tablets without any indication of flurazepam. Although a metabolite, desalkylflurazepam is active with a half-life of 47-100 hours. Cases positive for desalkylflurazepam from 2000 to 2020 were reviewed to determine the frequency of detection with flurazepam or other metabolites compared to detection on its own. Desalkylflurazepam concentrations were determined in 12 antemortem (AM) blood samples from 2020. Case studies will be presented.

Objectives: To evaluate trends in desalkylflurazepam detection between 2000-2020, and determine how often a parent compound was not indicated. Concentrations of desalkylflurazepam in current AM blood samples were also examined.

Methods: Twelve AM bloods previously determined positive for desalkylflurazepam and available at the lab were quantified. A sample size of 0.25 mL was mixed with 0.05 mL of deuterated internal standard (nordiazepam-d5) then extracted by protein precipitation using 0.75 mL cold acetonitrile and centrifugation. The supernatant was aspirated using DPX® WAX tips to further remove impurities in the sample, then 0.05 mL was transferred to a vial with 0.80 mL of initial mobile phase. Quantitative analysis was performed by liquid chromatography-tandem mass spectrometry (LCMSMS). The LC used was a Waters Aquity UPLC with BEH C18 column using aqueous (A) and organic (B) phases of 100% water and acetonitrile, each with 0.1% formic acid. Initial mobile phase was 80% A and 20% B with a 0.4 mL/min flow rate utilizing a 10 minute gradient. The MSMS was a Waters XeVo-TQS in MRM ESI+ mode with two ion transitions. This method was previously validated with SWGTOX validation standards. The quantitative range was 5-250 ng/mL on a quadratic curve, weighted 1/x and was for research purposes only.

Results: Between 2000 and 2020, 132 cases contained desalkylflurazepam; 43 were AM bloods and 89 were postmortem bloods/tissues. Of the 132 cases, 50% had only desalkylflurazepam without indication of flurazepam. Nearly 20% of those desalkylflurazepam only cases were from 2020. Uniquely, 2020 was the only year where parent flurazepam was never detected. It was also the only year where more than 50% of desalkylflurazepam cases were in AM blood. In the 2020 AM blood cases, concentrations ranged from 10.4 ng/mL to greater than 250 ng/mL, with an average (median) concentration of 52.5 ng/mL (29.3 ng/mL). Other drugs detected included fentanyl, methamphetamine, THC and/or metabolites, benzoylecgonine, mitragynine, morphine, and other benzodiazepines. The other benzodiazepines were chlordiazepoxide and metabolites, 7-aminoclonazepam, clonazolam, etizolam, and flualprazolam. This differed from cases prior to 2020 where other benzodiazepines detected were all prescription drugs and metabolites. The seized tablets containing desalkylflurazepam were visually identified as "S|90|3" alprazolam tablets. However, alprazolam was not confirmed or indicated in any of the tested tablets. Desalkylflurazepam was confirmed by both gas and liquid chromatography with mass spectrometry.

Conclusion/Discussion: Desalkylflurazepam has been detected in Orange County, California fairly regularly since 2000. However, in most cases, flurazepam or a metabolite was also detected, indicating ingestion of parent drug. An unexpected change was observed in August 2020 where every case with desalkylflurazepam detected lacked any indication of parent drug ingestion. If there is an indication it may be present in local jurisdictions, it may be prudent to add desalkylflurazepam confirmation to current testing protocols due to its activity.

POSTER

P-047: QuEChERS Extraction and LC-MS/MS Quantitation of Fentanyl Analogs and Metabolites from Liver Tissue to Support Medicolegal Death Investigations

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Background/Introduction: In medico-legal death investigations, the interpretation of toxicological findings is a crucial component to establish the cause of death of an individual. During the examination, the submission of blood, liver, and other biological specimens for toxicology testing is common practice. Due to its relevance and legal implications, the correct reporting of drug concentrations found in these tissues is of utmost importance for practitioners. However, when working with biological specimens, the analyst is prone to deal with tissues that are in various stages of decomposition. Also, natural processes like autolysis and putrefaction can affect the overall integrity of the specimen. To overcome this complexity, a toxicologist relies on extraction strategies that help to mitigate such challenges. The development of a quick, easy, cheap, effective, rugged, and safe (QuEChERS) extraction method is presented in this study as an alternative analytical extraction approach that improves the detection and quantitation of fentanyl analogs and metabolites from liver tissue. By its nature, the liver contains many lipids, proteins, salts and other impurities that can be problematic for target analyte detection. QuEChERS is ideal for tissue processing due to its powerful mechanism that allows for a direct extraction with an organic solvent in the first step followed by a dispersive-solid phase extraction as a second step. The combination of these two steps offered a great advantage to improve the detection capability of target fentanyl analogs using mass spectrometry tools.

Objectives: The overall goal of this project is to develop a comprehensive analytical approach that allows the quantification of fentanyl analogs and metabolites from complex biological matrices while removing the risk of cross contamination and reducing sample and solvent amounts.

Methods: For extraction, postmortem tissue was weighed out at 0.1 g into 1.7 mL centrifuge tubes and then 3 steel BB balls were added, acetonitrile, and QuEChERS extraction powder that contains magnesium sulfate and sodium chloride followed by dispersive-SPE clean up. The validation of the quantitation method for the 34 fentanyl analogs was performed with limit of quantitations (LOQs) of 0.05 or 0.5 µg/kg depending on the analyte and analytical measurement ranges (AMRs) of 0.05 to 10 µg/kg or 0.5 to 100 µg/kg depending on the analyte. An evaluation of a QuEChERS extraction procedure is presented herein this study as an alternative analytical method for efficient extraction and detection of fentanyl and fentanyl analogs. The evaluated parameters include selectivity, matrix effects, linearity, bias, precision, dilution integrity, and proof of applicability.

Results: For the 34 fentanyl analogs, ionization suppression or enhancement for the low ranged from -10.2% and 23.7% with an average of 4.9% and the high ranged between -7.1% and 11.0% with an average of 4.2%. The average bias for the lower limit of quantitation was 6.9% and the average precision was 11.6%. The average bias at the low concentrations was -1.5% and the average precision was 9.9%. The average bias at a high concentration was -0.6% and the average precision was 7.6%. For proof of applicability, 22 liver specimens from authentic postmortem casework were quantitated for the 34 fentanyl analogs. All 22 samples contained at least one of the 34 analytes of interest; including fentanyl, 4-ANPP, norfentanyl, carfentanil, methoxy acetyl fentanyl, 4-fluoroisobutyryl fentanyl (FIBF), valeryl fentanyl, and acetyl fentanyl. The highest concentration detected was 541 µg/kg of FIBF and was detected in four specimens.

Conclusion/Discussion: The comprehensive extraction and LC-MS/MS method developed for analysis of liver tissue for fentanyl and analogs is precise, sensitive, and reproducible for complex forensic matrices such as liver tissue. The method was proven applicable to postmortem casework by quantifying 22 authentic postmortem liver specimens.

Keywords: QuEChERS, Fentanyl Analogs, Liver, Novel Psychoactive Substances, Mass Spectrometry

POSTER

P-048: **Quantitation of 22 fentanyl analogs by UHPLC-MS/MS**

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Background/Introduction: The United States is facing an opioid crisis that includes not only the abuse of prescription drugs but also synthetic opioids. According to the Centers for Disease Control and Prevention (CDC), rates of overdose deaths involving synthetic opioids other than methadone, but including fentanyl, increased by 10% from 2017 to 2018. Over 31,000 people died from overdoses involving these compounds in 2018 alone. The CDC, in collaboration with Cerilliant Corporation™, released a Traceable Opioid Materi-al™ Kit (TOM Kit™) consisting of 22 fentanyl analog compounds with matched carbon-13 and nitrogen-15 isotopically labeled internal standards for quantitation and confident identification. Here we present a method for quantitation of the TOM Kit compounds in urine that includes sample preparation by supported liquid extraction (SLE) and quantitation by selected reaction monitoring (SRM) on Ther-mo Scientific™ TSQ Altis™.

Objectives: Demonstrate the speed and sensitivity of the Thermo Scientific™ TSQ Altis™ Triple Quadupole mass spectrometer for fast and accurate analysis of 22 fentanyl-related compounds, including fentanyl, in urine for clinical research and toxicology.

Methods: All standards were contained in the Traceable Opioid Material™ Kit. Non-labeled standards were combined into a stock solu-tion and diluted in methanol and then prepared in human urine to create a 13-point calibration curve ranging from 0.01 ng/mL to 100 ng/mL. Quality control (QC) standards were prepared at a concentration of 1.0 ng/mL, 2.5 ng/mL, and 25 ng/mL in urine. A 25 ng/mL internal standard (IS) working solution was created by combining the carbon-13 and nitrogen-15 isotopically labeled standards and di-luting in water. The IS working solution was added later to the non-labeled standards, prior to sample preparation by supported liquid extraction (SLE), for absolute quantitation. Each of the standards in the 13-point calibration curve and the QC standards were prepared by SLE with the Biotage® ISOLUTE® SLE+400 plates prior to analysis, with elution by dichloromethane, drying down under nitrogen, and resuspending with water:methanol (90:10) (v:v). The samples were then transferred to HPLC autosampler vials. 10 µL of each sample was injected and then separated by a 7.5 minute chromatographic method using a Thermo Scientific™ Vanquish Flex™ UHPLC system, consisting of a binary pump, a column compartment, and an autosampler. The separation was performed on a Thermo Scientific™ Ac-cucore™ Phenyl Hexyl column (2.6 µm, 2.1 mm x 100 mm) maintained at 40 °C. Mobile phases consisted of 2 mM ammonium formate in water with 0.1 % formic acid for mobile phase A and a mixture of 2 mM ammonium formate in methanol: acetonitrile (50:50 v:v) with 0.1 % formic acid for mobile phase B. Compounds were detected on a Thermo Scientific™ TSQ Altis™ mass spectrometer equipped with a Thermo Scientific™ OptaMax™ NG ion source with a heated electrospray ionization probe. SRM scans were collected for one quantifier and two qualifier ions for all fentanyl analogs and isotopically labeled fentanyl analog standards.

Results: All 22 fentanyl compounds eluted chromatographically between 1.54 and 3.84 minutes with baseline separation of all iso-mers. Data was acquired and processed using Thermo Scientific™ TraceFinder™ 5.0 software. Quantitation by internal calibration was used for all 22 fentanyl compounds with each fentanyl standard having a corresponding stable-isotope-labeled internal standard. The TSQ Altis™ demonstrated LOQs ≤ 0.25 ng/mL for all but six out of the 22 fentanyl compounds, and all were quantitated linearly up to 100 ng/mL, having an R^2 above 0.99.

Conclusion/Discussion: A sensitive sub-8 minute method for detection of 22 fentanyl analogs in urine was developed using the Ther-mo Scientific™ TSQ Altis™ Triple Quadupole mass spectrometer for clinical research and toxicology purposes.

P-049: A Validated Qualitative Method for the Identification of Phenibut in Postmortem Blood and Urine by LC/MS/MS

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Background/Introduction: Phenibut (β-phenyl-γ-aminobutyric acid), a GABA$_B$ agonist, is not approved for medical use in the United States but is available through internet suppliers for self-medication or recreational use. Calls to poison control centers for phenibut have increased over the last five years, and there are many case reports of severe acute intoxications and withdrawals requiring hospitalization. Phenibut is not tested in routine toxicology settings but has been detected in targeted applications by liquid chromatography tandem mass spectrometry (LC/MS/MS). We present a postmortem case in which phenibut was positively confirmed in both blood and urine via a newly validated LC/MS/MS method.

Objectives: To validate a method for identification of phenibut in blood and urine using protein precipitation and LC/MS/MS.

Methods: Phenibut was incorporated into an existing assay for gabapentin, pregabalin, and baclofen. It was paired with gabapentin-D10 for an internal standard. Analytes and internal standards were isolated from 200 **μL** of blood and urine by protein precipitation with acetonitrile. Analytes were separated with gradient programming on an Agilent 1290 Infinity HPLC system using an Agilent Poroshell 120 EC-C18 column fitted with an Agilent EC-C18 guard column. Mobile phase consisted of 0.1% formic acid in water and 0.1% formic acid in acetonitrile. The composition started at 15% organic, held for 0.5 minutes, increased to 45% at 3.5 minutes, then 95% at 4.5 minutes held for 1 minute, returning to starting conditions at 6 minutes. Phenibut eluted at 1.4 min. Eluents were analyzed on an Agilent 6460 triple quadrupole mass spectrometer using electrospray ionization in positive mode monitoring two transitions per analyte, 180.1→145.0 and 180.1→117.0 for phenibut, in multiple reaction monitoring mode. The method was qualitatively validated following laboratory procedures adopted from ANSI/ASB Standard 036 Standard Practices for Method Validation in Forensic Toxicology. Parameters evaluated included limit of detection (LOD), carryover, ionization suppression/enhancement, and processed sample stability.

Results: LOD was administratively set and evaluated at 0.5 mg/L in blood and urine with samples meeting qualitative acceptance criteria. There was no carryover after samples fortified up to 40 mg/L. Overall suppression/enhancement was within ±25%. Processed samples were deemed stable for up to five days at room temperature. Phenibut was confirmed in both blood and urine of a 26-year-old male decedent whose autopsy findings were unremarkable. The decedent had a history of ethanol abuse, depression, anxiety, and three separate hospitalizations for suicidal ideation and ethanol rehabilitation; The most recent hospitalization occurred within four weeks of death. Packages labeled as phenibut were found on scene. No other toxicological cause of death was found through routine testing. Given the evidence found on scene and the routine test results, LC-time of flight MS data was retrospectively mined for the phenibut molecular weight and found to have a match. A neat standard was later obtained and analyzed via TOF to confirm retention time. This TOF finding prompted validation of a method to confirm phenibut.

Conclusion/Discussion: A qualitative LC/MS/MS method for the identification of phenibut in blood and urine was validated and used in postmortem casework. Although phenibut was confirmed in the postmortem samples, the cause and manner of death were ruled undetermined. Previous literature has described acute intoxication as well as severe withdrawal events however in most cases phenibut was not analytically confirmed.

POSTER

P-050: A Validated Method for the Quantification of Desmosine in Plasma, Urine, and Formalin-Fixed Autopsied Lung Tissue Specimens by LC–MS-MS

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Background/Introduction: Desmosine is a unique crosslink of elastin; synthesis of elastin crosslinks gives rise to elasticity in this tissue. As desmosine is only found in elastin, it can be consistently isolated and quantitated after elastin degradation in certain diseases. However, there have been no prior attempts to use LC–MS-MS to determine desmosine concentrations in formalin-fixed autopsied lung tissue. We describe a method for the quantitation of desmosine in formalin-fixed autopsied lung tissue specimens using LC–MS-MS to determine its validity as a biomarker for elastin degradation and for assessing respiratory status during medicolegal investigations. This method is suitable for use in forensic laboratories because validation was performed on instrumentation routinely used in forensic labs. This method is also useful in the pursuit of establishing the cause and manner of death where no apparent gross lung pathology may be present at autopsy, as desmosine concentrations provide insight into the relationship between the loss of elastin and the extent/progression of lung disease.

Objectives: To develop and validate an LC–MS-MS method for the quantitation of desmosine in plasma, urine, and formalin-fixed autopsied lung tissue specimens.

Methods: Lung specimens were obtained from a hamster animal model exposed to either room air or cigarette smoke to establish proof of concept. Donor plasma and urine were obtained from UTAK Laboratories. A sample size of 1 mL (plasma, urine, or tissue homogenate) was used to prepare calibrator, QC, and authentic samples. Extraction of desmosine and desmosine-D_4 was facilitated by SPE utilizing Styre Screen BCX Extraction Columns obtained from UCT, with extracts reconstituted in 200 µL of a 95:5 mixture of water and methanol. Following SPE, the extracts were injected onto an Agilent 1260 LC equipped with a Poroshell 120 EC-C18 column (3.0 mm x 50 mm, 2.7 µm) utilized for the chromatographic separation of desmosine and desmosine-D_4. Mobile phases were: 0.01% formic acid, 5 mM ammonium formate, and 5 µM medronic acid in Milli-Q water (solvent A) and 0.01% formic acid and 5 µM medronic acid in methanol (solvent B). The mobile phase flow rate was 0.25 mL/min and the instrument injection volume was 2.5 µL. The total chromatographic run time was 3 min. Mass spectral identification was utilized with an Agilent Technologies 6460-triple quadrupole mass spectrometer with a dual jetstream electrospray source operating in positive ion mode. The multiple reaction monitoring (MRM) method monitored two transitions for desmosine and one transition for desmosine-D_4.

Results: One calibration curve using calibrators prepared in solid tissue, ranging 40–2000 ng/mL, was developed. Quality control specimens were prepared in plasma, urine, and solid tissue to verify proper calibration. The method was evaluated and produced acceptable validation results to ANSI/ASB standards. The method demonstrated a possible correlation between elastin breakdown, as measured by desmosine concentrations in the lung, and the degree of pulmonary airspace enlargement in a hamster model of COPD as visualized through histological analysis. Analysis of plasma, urine, and sputum samples collected from a previous COPD clinical trial demonstrated good agreement with a previously described method for desmosine quantification. Further studies are ongoing to determine its validity as both a biomarker for elastin degradation and a means of assessing respiratory status during routine medicolegal investigations, as well as in the real-time measure of COPD drug efficacy.

Conclusion/Discussion: We describe for the first time a practical, reliable, and validated LC–MS-MS method that can measure desmosine in plasma, urine, and formalin-fixed autopsied lung tissue specimens simultaneously on instrumentation commonly encountered in forensic laboratories. Research is currently ongoing to quantify desmosine in postmortem samples with an emphasis on formalin-fixed autopsied lung tissue from prior medicolegal death investigations.

POSTER

P-051: A mathematical model using long-acting injectable medications to estimate hair growth rate when establishing drug exposure history by segmental analysis

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Background/Introduction: Long-acting injectable medications (LAIs) can provide a time correlation to detected drugs in hair. LAIs are maintained for several months to years with documented dosages and timings. If variation of analyte concentration in hair can be correlated with known dosing intervals, then the growth rate of hair can be better estimated and time windows for past exposure more accurately calculated.

Objectives: To assess the feasibility of detecting analyte variation in hair incorporation of LAIs and to estimate the required precision to reliably detect this variation for an accurate estimation of hair growth rate and age of segments.

Methods: A mathematical model was created for plasma concentrations those established on LAIs. The model was fitted to common medications with dosing intervals of 2 weeks for zuclopenthixol (Clopixol®) and of 4 and 12 weeks for paliperidone (Invega®) and sequent results of segmental hair analysis. Monte Carlo algorithms were used to determine how accurately the dosing interval could be calculated in segment-lengths. 10-months' worth of simulated plasma levels were selected randomly from the second year onwards. The data was split to correspond to segments of one month (10mm), 2 weeks (5mm) and 10 days (3.3mm - the minimum length felt to be practicable) at a typical hair growth rate of 10mm/month. Gaussian noise was added to this data. In total, 50,000 hair segments were simulated per medication, segment length and coefficient of variation (CV). Accuracy was tested at ±0.5 and ±1 hair segments, CV of 1, 5 and 10% were simulated. Python's NumPy module simulated the data noise and the SciPy submodules were used for curve fitting (Optimize) and feature detection (Signal).

Results: The dosing interval was not accurately detected at any segment length for the 2-weekly depot. For the 4-weekly depot, the interval was accurately estimated at 2 and 3 segments per month. The 12-weekly depot was accurately detected at 1 and 2 segments per month. Results are summarized in Table 1.

Table 1: Configurations where over 75% accuracy was achieved.

Interval (Weeks)	Segments per Interval	% CV	Threshold Accuracy (segments)	Result (%)
12	1	10	1	99.6
12	1	10	0.5	80.8
12	1	5	1	98.4
12	1	5	0.5	89.4
12	2	5	1	84.9
12	1	1	1	100
12	1	1	0.5	97.6
12	2	1	1	100
12	2	1	0.5	98.4
12	3	1	1	100
4	3	10	1	98.4
4	3	10	0.5	88.3
4	2	5	1	75.7
4	3	5	1	99.5
4	3	5	0.5	95.9
4	2	1	1	96.6
4	2	1	0.5	88.4
4	3	1	1	100
4	3	1	0.5	100

Conclusion/Discussion: It may be possible to characterize hair growth rate for individuals already on LAIs using known pharmacokinetics and dosing intervals. Performance peaked around 3 segments per dosing interval. Based on typical hair growth rates, this is 10mm for the 12-weekly depot and 3.3mm for the 4-weekly depot. ±0.5 segment error at 3 segments per dosing interval corresponds to a ±16.7% error. Our model may play a potentially important role in drug facilitated crime hair analysis and could be implemented clinically and post-mortem. Nonetheless, further research to characterize the impact of other parameters and invitro testing will be needed before such a model gains wider acceptance.

P-052: Now You See It, Now You Don't – The Case of Vanishing Drugs in a Urine Drug Screen

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Background/Introduction: Urine samples are sometimes adulterated by persons when they are submitted for drug screening by enzyme immunoassay (EIA). Some means of urine sample adulteration can be detected by the first-generation urine sample validity tests (SVTs) but unscrupulous groups have invested significant amounts of time and money to sell products that defeat the first-generation urine SVTs. However, the second-generation (Validity Diagnostics - VDx GEN2-SVT™) urine SVTs can detect more classes of adulterants, including products that the donor consumes prior to submitting a sample for a urine drug screen that can mask the detection of drugs. The Validity Diagnostics GEN2-SVT™ reagent panel includes a dual-indicator pH reagent; a creatinine reagent with a decolorizing reagent to minimize interferences from hemoglobin; a specific gravity index reagent which measures total urinary sodium and potassium ions; an oxidant history reagent which measures the effect of an oxidizing agent; and, a reagent to detect urinary tract proteins.

Objectives: Identify potential sources of adulterants in "detox" drinks which may mask the detection of drugs by EIA. Evaluate the performance and applicability of second-generation urine SVTs for clinical and forensic use for high throughput SVT testing.

Methods: A coworker, legally using various drugs, volunteered to consume a "detox" drink and to donate urine samples over a period of time. The "detox" drink, Rescue Detox Ice Instant Cleansing Energy from Applied Sciences, allegedly contains B/C vitamins, sodium, potassium, protein, caffeine, creatine, inulin, and several botanical extracts and is marketed to mask drug detection when using EIA drug screens. The coworker followed the directions included with the "detox" drink and did not consume anything else during the urine collection period. Urine drug screens and first and second-generation urine sample validity tests were conducted on the urine samples.

Results: After consuming the "detox" drink, urine samples were collected at various times and were screened for drugs by EIA and were subjected to SVTs:

Time Zero and 25 minutes – Positive for amphetamines, cannabinoids and ethyl glucuronide

57 minutes – Positive for amphetamines and ethyl glucuronide
68 minutes – Positive for amphetamines
76 minutes and 118 minutes – NEGATIVE (these two urine samples passed the first-generation urine SVTs but failed the second-generation urine SVTs)
193 minutes and 261 minutes – Positive for amphetamines, cannabinoids and ethyl glucuronide

Conclusion/Discussion: This study was conducted to help determine the possible cause(s) of several failed urine SVTs, negative drug screens and follow-up positive LCMS results in a court-ordered probation drug testing program. The urine samples in question were flagged using the second-generation SVTs (VDx GEN2-SVT™) but were not flagged by the first-generation SVTs. After further investigation, the persons on probation informed the court that they were using the "detox" drink to pass the drug screens. The persons on probation also stated that while using drugs and the "detox" drink for several months, they did not experience any failed urine SVT tests when the laboratory was using the first-generation SVT tests. Based upon the studies in the probation drug testing program and the clinical drug testing program at my location, the use of the second-generations SVTs can increase the overall effectiveness of pre-analytical SVT testing by detecting many classes of adulterants. Because the second-generation SVT reagents can be used on automated clinical analyzers, they can be used without undue burden on drug testing laboratories. Urine specimens from donors failing SVTs with the VDx GEN2-SVT™ panel should have follow-up testing by LC/MS-MS.

P-053: The Impact of Changing Synthetic Cannabinoid Drug Trends in a Forensic Laboratory

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Background/Introduction: Novel psychoactive substances (NPS) continue to challenge forensic toxicology labs, requiring labs to update and re-validate methods and libraries in order to identify the most current analogs. This continuous updating and re-validation of NPS-specific methods can be resource intensive and a significant undertaking for a working laboratory when done in conjunction with casework and the validation of new methods.

Objectives: This presentation will demonstrate what is required from a high-throughput forensic laboratory to maintain a synthetic cannabinoid method amidst an ever-changing drug landscape.

Methods: The Toxicology Section at the Dallas County Southwestern Institute of Forensic Sciences (SWIFS) developed and validated a method for the detection of eighteen synthetic cannabinoids in blood in August of 2017. The analytes consisted of the most prevalent synthetic cannabinoids based on reports from drug monitoring sources (National Forensic Laboratory Information System (NFLIS), UNODC Early Warning Advisory (EWA)) at the time of the method's conception. Given the changing nature of NPS, not all the analytes comprised in this initial method are still prevalent and the method requires frequent updating to capture newly emerging compounds. To determine which new synthetic cannabinoids to add to the method, SWIFS relies on reports from NFLIS and the Center for Forensic Science Research and Education (CFSRE) in addition to findings from SWIFS' Drug Analysis Laboratory. The Synthetic Cannabinoids by LC-MS/MS assay is a qualitative, multiple reaction monitoring (MRM) method, and each new analyte addition requires a validation as described in Academy Standards Board (ASB) Standard 036. The validation includes evaluation of ionization suppression/enhancement, carryover, limit of detection, dilutions, processed sample stability, and interference studies. Prior to validation, new analytes are optimized and added into the existing acquisition method as "experimental" drugs, allowing the laboratory to tentatively identify the new analytes in casework and triage the affected cases; these cases can then be used as authentic samples in the validation. Upon completion of the validation, the samples are re-analyzed and reported.

Results: Since the validation of the initial method in 2017, SWIFS has updated the Synthetic Cannabinoids by LC-MS/MS method three times to add a total of six new synthetic cannabinoids reported in literature and/or identified by the Drug Analysis Laboratory (Table 1). During the third update, the decision was made to remove six analytes from the initial validation that SWIFS had not reported and are no longer prevalent based on local and national synthetic cannabinoid trends. As the third addition was nearing completion, the Drug Analysis Laboratory identified and reported 5-fluoro EDMB-PICA and 4-fluoro ABUTINACA, leading to a pending fourth update.

Conclusion/Discussion: Typically, once a method is developed, validated, and placed into production, it rarely requires re-visiting or updating. That is not the case with methods involving NPS, as these compounds are in constant fluctuation due to a number of factors such as popularity, scheduling, and/or availability. Therefore, these methods require frequent updates. In a production laboratory, this activity must be balanced with on-going casework and other validations. Updating these assays is necessary, however, in order to preserve their value and provide medical examiners and other customers with information regarding the most relevant drugs.

POSTER

Table 1. Analytes included in each Synthetic Cannabinoids by LC-MS/MS validation.

Initial Validation (08/2017*)	Addition #1 (05/2019*)	Addition #2 (08/2019*)	Addition #3 (02/2021*)	Addition #4 (TBD)
5F-AB-PINACA	5F MDMB-PICA	4F MDMB-BUTINACA	MDMB-4en-PINACA	5F EDMB-PICA
AB-FUBINACA	5F EDMB-PINACA		4F MDMB-BUTICA	4F ABUTINACA
AB-PINACA			5F EMB-PICA	
ADB-FUBINACA				
AB-CHMINACA			**Removal of:**	
MAB-CHMINACA			UR-144	
5F-AMB			XLR-11	
MMB-FUBINACA (FUB-AMB)			NM2201	
5F-ADB			PB-22	
5F-PB-22			5F-PB-22	
MDMB-FUBINACA			FUB-PB-22	
MDMB-CHMICA				
FUB-PB-22				
XLR-11				
PB-22				
UR-144				
NM2201				
JWH-018				
Internal Standard: AB-CHMINACA D_4				

*Month/Year validation report was completed/signed and method put into production

P-054: Qualitative Analysis of Drug Impregnated Paper Samples from England and Wales Prisons in 2019 and 2020

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Background/Introduction: Novel psychoactive substances (NPS) provide the same effects as classic drugs, such as cannabis, cocaine, and amphetamine. NPS are often smuggled into prisons by mail in the form of impregnated letters. The misuse of drugs in UK prisons is endemic, leading to violence, aggression, and disruptive behavior among prisoners.

Objective: The current research was conducted in order to develop a qualitative method to identify the variety of emerging novel psychoactive substances impregnated onto paper samples sent to prison inmates. The aim was to help rapid detection and identification, enabling the scope of the problem to be established.

Methods: From each piece of paper believed to be impregnated with drugs, approximately 1 cm square of paper was cut. Samples were placed into separate 1.5mL Eppendorf tubes with 1mL of 50% (v/v) methanol in LC-MS-grade water. Extracts were prepared from the samples by vortex-mixing (30 min). A mobile phase blank was injected between the analysis of each extract to check for carryover. Extracts were screened for NPS and other compounds using an Agilent Technologies 1290 Infinity II – 6545 Q-TOF LC/MS instrument with electrospray ionization in positive ion mode. It uses an Agilent Eclipse Plus C18 1.8 mm 2.1 x 100 mm column, maintained at 40°C. Drug separation was performed over a total of 13 min using a simple linear gradient of water (A), and methanol (B), both contained 0.01% (v/v) formic acid in 5 mmol/L ammonium formate, at a flow rate of 400 µL/min. Sample injection volume was 0.2 µL.

Results: A drug database which consists of more than 200 samples was created and utilized for the identification of unknown substances usually seen in forensic laboratories. 332 samples collected in 2019 from seven UK prisons and 563 samples collected in 2020 from four UK prisons were screened with this method. Synthetic cannabinoids were the most common drug category detected in prison letter samples in 2019. Miscellaneous and SCRAs are the most common drug category in 2020 samples.

Table 1 *Percentages of the drug types in total in 2019 & 2020 in the UK prisons*

2019 Total		Drug Types	2020 Total	
Total	**Percent**		**Total**	**Percent**
0	0%	Anabolic steroids	0	0%
0	0%	Cannabis	0	0%
3	0%	Nicotine	35	2%
26	2%	Abused prescription drugs	37	3%
38	3%	Other medication	35	2%
564	51%	Synthetic cannabinoids	349	25%
75	7%	Miscellaneous	359	25%
45	4%	No drugs detected	256	18%
247	22%	Class A	309	22%
111	10%	Class B (excl. cannabis & SCRAs)	37	3%
1109	100%	Total	1417	100%

Conclusion/Discussion: This research is a confirmation of the findings of another publication in this area (Ford and Berg, 2018) and confirms that NPS being brought into UK prisons via drug impregnated letters posted to prisoners still remains a problem. With analytical confirmation of drug impregnated letters sent to prisoners, which include NPS, we have generated qualitative data to assist detection and identification information and have demonstrated that novel psychoactive substances are still entering UK prisons in this way.

POSTER

P-055: Detection of Mitragynine and 7-Hydroxymitragynine in Keratinized Specimens

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Background/Introduction: *Mitragyna speciose*, known as kratom, is a native tropical tree to southeast Asia, with leaves that contain compounds that can have psychotropic effects. The species has been used in herbal medicine and is used in religious traditions. The leaves of the kratom plant can be chewed, brewed in tea, smoked, or eaten in food. However, most people take kratom as a pill, capsule or extract. Two compounds in kratom leaves, mitragynine and 7-hydroxymitragynine, interact with mu-opioid receptors in the brain, producing sedation, pleasure, and decreased pain, especially when users consume large amounts of the plant. When kratom is taken in low to moderate doses it produces stimulant effects. The legality of the plant versus the legality of the specific analytes is becoming increasingly complicated as states take different legal actions as well as the federal government.

Objective: The objective of this study is to develop and validate a method for the detection of mitragynine and 7-hydroxymitragynine in keratinized specimens using a Laser Diode Thermal Desorption Tandem Mass Spectrometry (LDTD-MS/MS) screening analysis and a Liquid Chromatography Tandem Mass Spectrometry (LC-MS/MS) confirmation analysis.

Methods: Keratinized specimens (hair or nail) are weighed out (20 mg) and washed using acetone. The sample was homogenized in a Biospec Beadbeater-24. The specimens were sonicated in methanol and the supernatants were subjected to a solid phase extraction procedure. The dried extracts were reconstituted with a methanol: deionized water solution containing 100 µg/mL of EDTA. Extracts (8 µL) were then spotted into a LazWell 96 Plate [Phytronix, Toronto, Canada] and dried prior to analysis on the LDTD-MS/MS. Presumptive positive specimens were confirmed through a second aliquot (50 mg), washed, homogenized, sonicated in methanol. The supernatant was filtered prior to analysis by LC-MS/MS.

Results: The Standard Practices for Method Validation[1] was followed. The LDTD-MS/MS analysis had a cutoff of 100 pg/mg, with a limit of detection of 30 pg/mg. Both the extracts and dried LazWell plates were stable for 48 hours, and no carryover or obvious interferences were observed during the validation. Significant ion suppression was observed for 7-hydroxymitragynine; however, significant ion enhancement was observed for mitragynine. Authentic specimens were analyzed and presumptive positive specimens were forwarded to LC-MS/MS for confirmation. The confirmation method was validated with a cutoff of 100 pg/mg and a limit of detection of 5 pg/mg for 7-hydroxymitragynine and 3 pg/mg for mitragynine. Precision and accuracy were challenged at 40 pg/mg (Low), 125 pg/mg (Mid) and 1000 pg/mg (High), following our standard in-house procedure for setting up assays, and results were acceptable. Extracts were stable for up to 7 days, there was significant ion suppression, >25% for both analytes and no obvious interferences or carryover were observed. Linear range was determined to be between 40 pg/mg and 2000 pg/mg. All authentic specimen analyzed showed 100% correlation with the screening analysis.

Discussion/Conclusion: Kratom has found an increased use among people who are looking at alternatives to traditional opioids. The validated method described here allows accurate identification of usage of kratom, allowing for objective proof that can be used by rehab and diversion programs to intervene and provide more successful outcomes. The legality of the plant is in flux, with four states making it legal and six states banning it, while other states have either enacted age restrictions or are waiting before enacting any regulation. On a federal level, the FDA has no approved use for kratom, while the DEA has listed kratom as Drug and Chemical of Concern.

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1   ANSI/ASB Standard 036, First Edition, Standard Practices for Method Validation, 2019

P-056: Designer Benzodiazepine Positivity and Co-Positivity in Urine Specimens Received from December 2020 to April 2021

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Background/Introduction: Designer Benzodiazepines (DBZD) are synthetic substances that produce similar pharmacological effects as prescription benzodiazepines, though are not approved for use in the United States of America (USA). According to the Drug Enforcement Administration (DEA) 2020 Annual Emerging Threat Report, a 157% increase in identifications of DBZD was observed in 2020 compared to 2019. Flualprazolam and etizolam accounted for approximately 81% of all DBZD identifications followed by clonazolam and flubromazolam at approximately 11 and 3%, respectively. Flualprazolam has been identified in counterfeit alprazolam tablets and was noted in the DEA 2020 National Drug Threat Assessment as a cause for overdoses across the country. Use of DBZD will go undetected when patients are only tested for traditional benzodiazepines via mass spectrometry.

Objectives: To evaluate DBZD positive urine specimens to determine prevalence of specific DBZD, co-positivity with other drugs/drug classes, and patient demographics including age and sex.

Methods: This study was IRB approved. DBZD were analyzed as part of a larger Novel Psychoactive Substances (NPS) panel. DBZD analyzed included bromazolam, clonazolam, 8-aminoclonazolam, diclazepam, delorazepam, etizolam, alpha-hydroxyetizolam, flualprazolam, alpha-hydroxyflualprazolam, flubromazepam, flubromazolam, alpha-hydroxyflubromazolam, nitrazolam, phenazepam, and 3-hydroxyphenazepam. From December 2020 to April 2021, 28,900 samples requesting NPS testing were submitted from 36 states in the continental USA. Prior to analysis, DBZD were extracted from hydrolyzed urine using a liquid-liquid extraction followed by evaporation and reconstitution in mobile phase. Samples were injected onto a liquid chromatography/tandem mass spectrometry (LC-MS/MS) instrument consisting of a Shimadzu Prominence HPLC and Sciex API 4000 MS/MS. The mass spectrometer was operated in positive electrospray ionization mode for scheduled multireaction monitoring (sMRM) analysis. Analytes were chromatographically separated on a biphenyl column. Results were reported qualitatively with detection limits ranging from 1-5 ng/mL. Samples positive for DZBD were evaluated for co-positivity with other drugs/drug classes. Co-positive analytes were analyzed by separate validated methods.

Results: In the five month study period, the percent DBZD positivity by month with respect to the number of DBZD tests ordered ranged from 1.3-1.8%. 396 specimens were positive for DBZD with 51% from male donors and 49% from female donors. The median patient age was 37 and ranged from 18-76. Clonazolam (8-aminoclonazolam), etizolam (alpha-hydroxyetizolam), flualprazolam (alpha-hydroxyflualprazolam) and flubromazolam (alpha-hydroxyflubromazolam) were evaluated as parent drug and metabolite. For these DBZD, a number of specimens contained metabolite only (clonazolam 79%, etizolam 65%, flualprazolam 33%, and flubromazolam 25%). 337 (85%) specimens were positive for a single DBZD with etizolam (51%), clonazolam (34%) and flualprazolam (9%) being the most prevalent. Some samples had multiple DBZD detected, including 47 (12%) that were positive for two DBZD with clonazolam and etizolam combination being the most prevalent (45%), and 11 specimens were positive for three DBZD with the combination of clonazolam, etizolam and flualprazolam being most prevalent (46%). One specimen was positive for four DBZD (clonazolam, etizolam, flualprazolam and bromazolam). The highest DBZD co-positivity was for buprenorphine at 61% followed by carboxy-THC (39%), prescription benzodiazepines at 38% (44% clonazepam, 36% alprazolam) and opiate antagonists (e.g. naloxone) at 38%. Other co-positivities of interest include fentanyl (28%), opioids (24%), methamphetamine (21%), gabapentin/pregabalin (20%), alcohol metabolites (17%), amphetamine (13%), cocaine (12%), designer opioids (8%), heroin (6%) and synthetic cannabinoids (3%).

Conclusion/Discussion: An increase in use of DZBD has been observed in recent years. This study demonstrated use of DBZD with other benzodiazepines, opioids, medications used to treat opioid addiction, and other CNS depressants which represent risk of overdose and death. Use of DBZD will go undetected with traditional mass spectrometric benzodiazepine testing therefore, testing for DBZD in at risk populations may provide a more complete picture of an individual's substance use history and allow for more informed care.

POSTER

P-057: Fentanyl with a dash of Xylazine?

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Background/Introduction: Xylazine first gained fame by being detected with heroin and cocaine; and it is again garnering attention in combination with fentanyl. It is a veterinary anesthesia sold as Rompun® by Bayer (Leverkusen, Germany) and was first introduced in 1962. The sedation effects and respiratory depression on the central nervous systems come from xylazine acting as an α_2 adrenergic receptor agonist. Common effects include sedation, analgesia, and muscle relaxation in animals. Previous attempts were made to have xylazine approved for human consumption but were unsuccessful due to concerning side effects like hypotension and bradycardia. Although the drug was approved for veterinary use, xylazine is still desirable by recreational users and commonly used as an adulterant to prolong desired effects. The possible synergistic effects of xylazine with fentanyl may result in incorrect diagnoses of overdose cases and often lead to adverse consequences.

Objectives: To bring awareness to the current trend of detecting xylazine in combination with fentanyl.

Methods: Method validation followed requirements of ANAB and ASB guidelines. Samples were analyzed by a Sciex X500R with Shimadzu Nexera Prominence LC with Phenomenex Kinetex Phenyl Hexyl, 2.6 μm, 50 x 4.6 mm column with Phenyl Guard column. Organic solvent (B) was 100% methanol with 0.1% formic acid and aqueous solvent (A) was 100% ultra pure water with 0.1% formic acid. LC method with flow rate of 0.7 mL/min started at 90% A, 10% B, linear ramp to 7.5 minutes at 2% A and 98% B, hold till 8.5 minutes. Then, a linear ramp to 90% A, 10% B to 8.6 minutes and hold till 9.5 minutes. Following the addition of 1.5 mL of the internal standard in cold acetonitrile, the samples, standards, and blanks (0.5 mL) were briefly vortexed and centrifuged for 10 minutes. The supernatant was then transferred and dried under heated air. Samples were reconstituted with 0.5 mL of initial mobile phase and transferred by glass pipette into vials. Xylazine has a limit of detection at 5 ng/mL for blood and urine samples.

Results: Xylazine was validated using the Orange County Crime Lab's (OCCL) LC-QTOF screening method in August of 2018. From then to March 2021, xylazine was confirmed in 51 cases in various sample types: antemortem blood (22 cases), postmortem blood (28 cases), and urine (1). An uptick of xylazine detection was seen from 2018 (3 cases) to 2020 (25 cases); a 733% increase within three years. With every case of xylazine, fentanyl was detected; additionally, 4 cases detected heroin and 1 case had cocaine. The average concentration of fentanyl was 0.0491 mg/L (median: 0.0218, range: 0.0013 – 0.303). Other common drugs found were 4-ANPP in 44 cases, norfentanyl in 39 cases, and methamphetamine in 30 cases. The Seized Drug section also detected xylazine in 51 case submissions of powder (45 cases), ethanol rinsate (2 cases), tar (4 cases), syringes (2 cases), and counterfeit pills (3 cases). There were 5 cases where toxicology testing was performed on postmortem central blood samples with additional evidence submitted for Seized Drugs analysis. All 5 blood samples detected fentanyl, norfentanyl and xylazine. Evidence types submitted were foil, powder, and a syringe. Toxicology testing found fentanyl (5 cases), 6-MAM (2 cases) and morphine (1 case). Cause of death of all 5 cases was deemed to be acute poly-drug intoxication, combined effects of fentanyl, and other drugs.

Conclusion/Discussion: Although previously known to be paired with heroin, cocaine, or both, our recent search from 2018 to March of 2021 showed that xylazine has been detected with other substances; notably fentanyl. Fentanyl alone can result in coma and/or death, without the consideration of other side effects caused by adulterants.

POSTER

P-058: Advanced search tools for identification of novel fentanyl analogs detected by HRAM Orbitrap mass spectrometry

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Background/Introduction: Deaths attributed to synthetic fentanyl analogs rose 10% from 2017 to 2018, likely driven by illicitly manufactured fentanyl analogs. Laboratories have established methods for common fentanyl analogs and other clinically relevant opioids, but the list of new illicit fentanyl analogs is rapidly expanding and can be challenging for laboratories to confirm without certified reference standards. Therefore, the Centers for Disease Control and Prevention (CDC) in collaboration with Cayman Chemical© released a Fentanyl Analog Screening Kit (FAS Kit) and Emergent Panels (FAS V1-V4) to provide standards to laboratories for screening of fentanyl analogs. Here we present a workflow that uses Thermo Scientific ™ Compound Discoverer™ 3.2 software to enable identification of unknown fentanyl analogs before they become available in a newly released emergent panel.

Objectives: Identify novel unknown fentanyl analogs by compound class score and molecular networking search tools available in Compound Discoverer™ 3.2 software. Utilize an MS^2 library for 212 fentanyl analogs to establish class-based and structural similarity rules for fentanyl analogs and correctly identify unknown standards spiked into urine samples.

Methods: A local MS^2 library was created in Thermo Scientific™ mzVault™ software for 212 fentanyl analog compounds contained in the FAS Kit and Emergent Panels v1-v3 in. MS^2 scans and retention times were obtained for all 212 fentanyl analog compounds using a chromatographic method of 15.5 minutes and a Thermo Scientific™ Vanquish Flex™ UHPLC system. The separation was performed on a Thermo Scientific™ Accucore™ Phenyl Hexyl column (2.1 mm x 100 mm, 2.6 μm) maintained at 40 °C. Mobile phases consisted of 2 mM ammonium formate in water with 0.1% formic acid for mobile phase A and a mixture of 2 mM ammonium formate in methanol: acetonitrile (50:50 v:v) with 0.1% formic acid for mobile phase B. Compounds were detected on a Thermo Scientific™ Orbitrap Exploris™ 120™ mass spectrometer. Full scan MS was used for screening and semi-quantitation while targeted MS^2 by data-dependent analysis was used for confirmation. A targeted mass inclusion list containing 212 fentanyl compounds with expected retention times and accurate m/z was used as a preferred targeted list. The mass spectrometer acquired MS^2 data for those in the preferred targeted list when present but deferred to the most intense unknown precursor ion when no known fentanyl analogs were present, enabling retrospective analysis of unknowns. The first 30 compounds of FAS Kit emergent panel v1 was spiked into urine and were analyzed as unknowns. The compounds in this emergent panel were not in the mzVault library and served as a test for the unknown discovery search tools found within Compound Discoverer software.

Results: Data was processed in Compound Discoverer with the accurate m/z and RT defined in a targeted search and with MS^2 provided as the in-house library to search against. All data was searched with the Thermo Scientific™ mzCloud™ cloud-based mass spectral database, which curates real-time compound additions, for MS^2 matches. The compound class scoring search was used to detect compounds against a set of five fragment ions known to be indicative of fentanyl compounds. The molecular networking feature was used to investigate possible relations between fentanyl compounds and unknown fentanyl compounds.

Conclusion/Discussion: Novel unknown fentanyl opioids were simulated by analyzing the first 30 compounds in emergent panel v1 from CDC as unknowns by Orbitrap Exploris 120™ mass spectrometer and data analysis in Compound Discoverer for advanced novel unknown compound detection and discovery. The combination of HRAM MS^2 data acquisition by Orbitrap technology and the of Compound Discoverer provided discovery workflows needed for a quickly expanding list of fentanyl analogs returned a positive identification for out 21 of 30 compounds. Future work will include data acquisition and analysis of emergent panel v4 as an unknown for retrospective analysis.

POSTER

P-059: Analysis of Suspected Opioid Overdose Samples for Fentanyl and Fentanyl Related Analogues using High Performance Liquid Chromatography Tandem Mass Spectrometer (LC-MS-MS) and High Performance Liquid Chromatography Quadrupole Time of Flight Mass Spectrometer (LC-QTOF-MS)

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Background/Introduction: The opioid crisis continues to grow resulting in a large number of fatal and nonfatal overdoses each year in the United States. Increasing use of fentanyl and fentanyl related analogues (fentalogues) poses a significant risk of fatal overdose due to their high potency and unpredictable pharmacokinetics. Fentanyl is a synthetic opioid primarily prescribed for pain management and anesthesia procedures but is also produced and used illegally, often mixed with other drugs. As part of the South Carolina (SC) statewide response to the opioid crisis, the SC Public Health Laboratory (PHL) began a pilot study to test specimens for fentanyl and fentanyl analogues from suspected opioid-related emergency department visits from across the state.

Objectives: The objective was to monitor exposure to fentanyl, fentanyl related analogues and other drugs-of-abuse in order to provide timely information on emerging drug trends for opioid biosurveillance across the state to effectively combat the opioid crisis. Herein, we will provide a comparison of the quantitative and qualitative methods for analysis of suspected opioid overdose samples for fentanyl and fentanyl related analogues using LC-MS-MS and LC-QTOF-MS, respectively.

Methods: The analytes were extracted from the urine test specimens using supported liquid extraction (SLE) and analyzed quantitatively on a High Performance Liquid Chromatography Tandem Mass Spectrometer (LC-MS-MS) and qualitatively on a High Performance Liquid Chromatography Quadrupole Time of Flight Mass Spectrometer (LC-QTOF-MS). The LC-MS-MS quantitative method was validated for 16 fentanyl related analytes, these include: Fentanyl, Carfentanil, 3-Methyl fentanyl, Acryl fentanyl, Valeryl fentanyl, Cyclopropyl fentanyl, Furanyl fentanyl, para-Fluorobutyryl fentanyl, Butyryl fentanyl, para-Fluoro fentanyl, β-Hydroxythio fentanyl, Acetyl fentanyl, U-47700, U-48800, U-49900 and 4-ANPP. The qualitative method on the LC-QTOF-MS was validated to include 20 analytes in the quality control solutions, the additional analytes in the qualitative method include: fentanyl analogues, Methoxyacetyl fentanyl and Remifentanyl; metabolites, Norfentanyl and Norcarfentanyl; and a fentanyl antidote, Naloxone. Currently, we are building our in-house library on the LC-QTOF-MS to expand the testing to 200+ synthetic opioids, the majority of which are fentanyl analogues, and other drugs of abuse.

Results: The SC Public Health laboratory has analyzed over 500 residual urine samples for the analysis and detection of 20 different fentanyl analogues. Of these, about 20% tested positive for fentanyl or fentanyl analogues. Overall, there was a good correlation between the analysis results on both methods and instruments. Due to the better sensitivity of the LC-MS-MS and lower limit of detection of the quantitative method, it was able to detect positive samples containing as low as 0.1 ng/ml of the analyte whereas the limit of detection varied from 0.5 ng/ml to 7 ng/ml for the 20 analytes on the LC-QTOF-MS method. The most common detected analytes were fentanyl and 4-ANPP on both methods. Other analytes that tested positive in the submitted specimens include para-Fluorofentanyl, Acetyl Fentanyl, para-Fluorobutyryl fentanyl, Valeryl fentanyl, Butyryl fentanyl, Acryl Fentanyl, 3-Methyl Fentanyl, β-Hydroxythiofentanyl, Furanyl fentanyl, Carfentanil, U-47700 and U-48800.

Conclusion/Discussion: The LC-MS-MS provides better sensitivity and lower detection limits, whereas the LC-QTOF-MS can scan for more fentanyl analogues, allowing the laboratory to identify novel fentanyl analogues that are being used within affected communities. These novel fentanyl analogues can be added to the growing list of fentanyl analogues to help other public health laboratories and stakeholders recognize a new threat on the market. Specimens from opioid-related emergency department visits from hospitals around the state have resulted in an expansion of the biosurveillance of fentanyl analogue usage in a broader segment of the SC population. We continue to work towards partnering with other hospitals to provide a better understanding of the opioid epidemic within South Carolina.

POSTER

P-060: Evaluation of EtG and EtS alcohol markers in urine and oral fluid after hard kombucha consumption

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Background/Introduction: Kombucha is a fermented beverage made from tea, sugar, yeast, and bacteria and may be labeled as "non-alcoholic" only if the alcohol by volume content (ABV) of the finished product does not exceed 0.5%. A previous study determined that the alcohol markers ethyl glucuronide (EtG) and ethyl sulfate (EtS) were not detected in oral fluid but were present in some urine samples after consuming "non-alcoholic" kombucha. An alcoholic version (hard kombucha) with a %ABV similar to beer (4–7%) is also available. This study evaluates EtG and EtS concentrations in urine and oral fluid after consuming hard kombucha. Despite the increasing popularity of kombucha, limited studies exist examining alcohol markers in biological specimens following consumption of hard kombucha.

Objectives: The objective of this IRB-approved study (#IRB-2019-249) was to determine urine concentrations of EtG and EtS and oral fluid concentrations of EtS after controlled consumption of 16 ounces (oz.) of hard kombucha.

Methods: Nine subjects participated in this study, five males and four non-pregnant females. Participants provided urine voids (V_0) and oral fluid specimens (Oral-Eze™) (t_0) before consuming a single 16 oz. bottle of hard Kombucha (6.5% ABV) within 20 min. Oral fluid specimens were collected 10 min after the hard kombucha was consumed ($t_{0.5h}$) and at 1, 3, 5, 8, 10, 24, 32, and 48h relative to the start of drinking. Participants provided all urine voids for the first day and then the first urine void on days 2 and 3. Participants were asked to abstain from all alcohol consumption the evening before administration and during the study. Oral fluid specimens were screened and confirmed when positive for EtS (25 ng/mL cutoff) by liquid chromatography tandem mass spectrometry (LC-MS/MS). Urine specimens were analyzed by immunoassay (IA) (500 ng/mL EtG cutoff) and LC-MS/MS for EtG (500 ng/mL cutoff) and EtS (100 ng/mL cutoff). Urine concentrations were not normalized for creatinine. Urine specimens were considered positive if EtS was present with or without EtG above these limits; urine EtG-only samples were not considered as positive. Kombucha beverages were also evaluated by headspace gas chromatography to determine %ABV.

Results: Kombucha bottles contained 4.0–4.5% ABV, which was significantly lower than the labeled 6.5%. Out of the 90 oral fluid specimens collected, 28 samples had EtS detected above the LOD (10 ng/mL). EtS was above the cutoff (25 ng/mL) in 3 subjects 27.1, 29.8, and 50.6 ng/mL occurring at 1, 3, and 3h after start of drinking, respectively. EtS was detected >LOD for up to 8h in 2 participants. In urine, EtG and EtS were detected by LC-MS/MS at median (range) concentrations of 3,381 ng/mL (510–70,250) and 770 ng/mL (104–15,364), respectively. When comparing IA-positive EtG specimens to LC-MS/MS, there were 48 true positive, 24 true negative, one false negative (510 ng/mL EtG, 188ng/mL EtS), and two false positives (104 ng/mL EtG, 65 ng/mL EtS; 457 ng/mL EtG, 229 ng/mL EtS). Urine specimens were all negative for EtG and EtS by the end of the 48h study.

Conclusion/Discussion: Although the alcohol content of the hard kombucha was lower than advertised, consumption of a single 16 oz. bottle of hard kombucha yielded positive results for alcohol markers in both oral fluid and urine. EtS was detected for up to 8h in oral fluid and EtG/EtS detected for up to 24h in urine. These results demonstrate the importance kombucha awareness for clinicians, patients, and medical review officers in abstinence-monitoring testing situations like workplace drug testing. These data also demonstrate the importance of confirmatory urine testing, particularly to include EtS.

POSTER

P-061: Profiling Trace Element Contaminants of Toxicological Interest in Commercially Available CBD Tincture Oils

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Background: There is legitimate concern over the rise in chronic health issues related to metals contamination in CBD infused products. Hemp, a hyperaccumulator plant, boasts the high biomass, long roots, short life cycle, and genetic structure ideally suited for absorbing and accumulating heavy metals. The situation is exacerbated by the popularity of these products. Sales to U.S. consumers in the form of pharmaceuticals, dietary supplements, foods, and other consumables are expected to grow >20% annually and reach ~$25 billion dollars by 2025. As demand outpaces supply, hemp plants are being sourced from outside of the United States, in some cases from countries having questionable environmental policies. Yet despite these facts, the regulatory environment is highly fragmented with inconsistent product standards and testing protocols from one state to another.

Objectives: The objective of this study is to document the trace metal profile in a sampling of commercially available full spectrum CBD tincture oils with the aim of:

- Identifying and quantifying elemental contaminants of toxicological interest
- Assessing truth in labelling relative to those metals that are regulated.
- Assessing the adequacy of the scope of state mandated heavy metals testing requirements

Methods: A "market basket" study of 18 hemp oils was conducted. The samples were full spectrum CBD oils having a potency of 25 – 50 mg/mL CBD, and naturally flavored (although this was not possible with all producers). Those variables which could not be controlled given the guidelines presented include method of solvent extraction (supercritical fluid vs ethanol extraction), final solvent (MCT, olive oil or hemp oil), and the source of the hemp from which the CBD was derived. Samples were prepared using microwave digestion with each batch including a digestion blank and QC standard followed by ICP-MS analysis for >40 elements. Three replicate digests of each sample were analyzed. Three different lots of material were purchased so that temporal, i.e., lot to lot variation, could be studied.

Results: The majority of elements monitored (including Hg, As, and Cd) were either below the limit of quantification or below regulated levels. Only the samples diluted in hemp oil were found to contain levels of Mn as high as 0.73 +/- 0.06 ppm. For reference the FDA has established that the Mn concentration in bottled drinking water should not exceed 0.05 ppm and the WHO drinking water quality guidelines for Mn are 0.4 ppm. Two samples were found to contain elevated levels of lead, 1.59 +/- 0.14 ppm and 3.64 +/- 0.08 ppm, nearly 3 to 7 times state regulated levels of lead in cannabis products. The published certificate of analysis for the sample containing 1.59 ppm of lead indicated that the lead concentration was below the regulated level at the time the product was tested. The published certificate of analysis for the sample containing 3.62 ppm of lead did not indicate that any metals testing was performed on the final product.

Conclusion/Discussion: The potential exists for heavy metal contamination in consumer products despite current regulations which can be ambiguous and fragmented. A question exists as to whether the elevated lead levels are the result of poor testing protocols and hence the need for standardized testing or if there might be other sources of contamination post testing i.e. contamination from packaging material. The study is still in progress to assess the variability between lots of the sample products from each given manufacturer.

P-062: Suicide by Tetrahydrozoline in North Carolina

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Background/Introduction: Tetrahydrozoline (also tetryzoline) is a compound in the class of imidazole derivatives that can be found in common over-the-counter eye drops (Visine, Tyzine, Murine). It has a high bioavailability that is distributed throughout the body including crossing the blood-brain barrier. When used for approved ophthalmic use, peak serum concentration averages 0.22 ng/mL. It has been documented to have a half-life of 6 hours in adults and 2-4 hours in children. As an alpha-2 agonist, it causes vasoconstriction of the blood vessels in the eye reducing redness. It is also an imidazoline-1 agonist which when taken orally can cause initial hypertension followed by hypotension leading to respiratory depression, coma and death. It has been documented in several pediatric accidental poisonings that with medical support, patients can recover from oral exposure. In scientific forensic literature, it has been identified as a substance used in drug facilitated sexual assault (DFSA) and more recently it has been implicated as a means to commit homicide. The North Carolina Office of the Chief Medical Examiner (NC OCME) presents a case of intentional ingestion of several bottles of Visine Original Redness Relief eye drops leading to death by suicide. Case study: In 2019, a 45-year-old male with a history of quadriplegia was found unresponsive in his wheelchair in his van parked in a hospital parking deck with 9 empty or partially empty bottles of Visine (0.05% tetrahydrozoline preparation). One bottle was up-ended in a large tumbler in the cupholder in the front seat with liquid still remaining in the cup, while other bottles were scattered in the front and back seats. The exact quantity of eye-drops ingested is unknown. A note requesting to "see text message" was found with the body, a suicide message was discovered on his phone. Autopsy findings include cardiomegaly, mild coronary artery atherosclerosis and pulmonary edema.

Objectives: This presentation will make attendees aware of the possible deadly effects of the oral ingestion of tetrahydrozoline and provide analytical information to assist practitioners with future cases.

Methods: Tetrahydrozoline can be detected in two of the routine screening assays performed at the NC OCME. In the organic base screen, a liquid-liquid extraction is followed by analysis by both GC-MS, where it can be identified by its mass spectrum, and GC-NPD, where it elutes directly after the internal standard, alphaprodine, with a similar retention time as caffeine, as compared with a certified reference. The second targeted screen involves protein precipitation with subsequent analysis by Orbitrap LC-MS.

Results: Tetrahydrozoline was identified in the organic base screen in both central blood and urine specimens from the decedent. The concentration of tetrahydrozoline in peripheral blood was found to be 0.13 mg/L, determined by a reference laboratory.

Conclusion/Discussion: This case from North Carolina documents a rare suicide by oral ingestion of eyedrops. Tetrahydrozoline is a compound that should be taken seriously by the forensic community as an agent that can cause harm, in both the DFSA and postmortem arenas.

POSTER

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P-063: Fatality from 2-Methyl AP-237 (2-methyl buccinazine), a novel synthetic opioid drug gaining recreational popularity

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Background/Introduction: 2-methyl AP-237 is an analogue of AP-237 (bucinnazine) which was originally synthesized in Japan in the 1970's as an analgesic compound and is still reportedly used as a therapeutic agent internationally. Structurally distinct from fentanyl and its derivatives, 2-methyl AP-237 is a synthetic opioid which has commonly been identified in seized material in Europe and the United States since early 2019. Neither 2-methyl AP-237 or AP-237 are currently scheduled in the United States but are classified as novel psychoactive compounds of the non-fentanyl opioid variety. As recreational use is on the rise, more recent studies have addressed important pharmacological questions including opioid receptor activity and metabolism not originally investigated decades ago.

Objectives: Present the first known case study corroborating solid dose material and blood findings that resulted in death after ingestion of 2-methyl AP-237.

Methods: The case presented is that of a 29-year-old male with a history of illicit substance abuse and mental health issues who was on house arrest for a narcotics DUI at the time of the incident. He was found unresponsive in his home after failing to respond to an in-home alcohol monitoring device and was pronounced deceased after resuscitation attempts. Law enforcement located drug related materials in close proximity, including a white substance, a scale, and other paraphernalia. Prescription medications found on scene included alprazolam, citalopram, and quetiapine. The autopsy was performed at the Johnson County Medical Examiner's Office with toxicology testing sent out to a reference laboratory. Postmortem toxicology cases submitted to the laboratory are screened for more than 300 prescription and illicit substances including designer opioids, synthetic cannabinoids, novel psychoactive drugs and novel emerging compounds by liquid chromatography quadrupole time-of-flight mass spectrometry (LC-QTOF-MS), with volatiles tested by headspace gas chromatography flame ionization detection (GC-FID). Presumptive positive results were confirmed and quantified by liquid chromatography tandem mass spectrometry (LC-MS/MS) using a separate specimen aliquot.

Results: Autopsy revealed moderate to marked pulmonary edema, constipation, and cerebral edema with uncal herniation. No significant natural disease to account for death was identified. Peripheral blood, urine, gastric, liver, brain and vitreous samples were collected during autopsy and blood, urine, and vitreous were submitted for toxicology testing. Femoral blood was positive for alprazolam (41.1 ng/mL), etizolam (19 ng/mL), and qualitatively positive for naloxone, caffeine, and cotinine. The urine was also positive for alprazolam and alpha-hydroxy alprazolam. The suspected white crystalline powder found on scene was tested and identified as 2-methyl AP-237 by the Johnson County Criminalistics Laboratory. Subsequently, a standard was purchased, and a special investigation was performed by the reference laboratory with qualitative identification of 2-methyl AP-237 in femoral blood using two separate aliquots. Additional method development and validation will be completed to determine concentrations in postmortem blood and disposition of drug in other biological matrices.

Conclusion/Discussion: While routine comprehensive toxicology testing identified a novel psychoactive compound, the concentrations of drugs present did not support cause of death in the absence of other anatomical findings. The analysis of suspected powder on scene revealed a novel opioid compound which guided additional toxicology testing in our specimens. This case emphasizes the benefit of investigative, pathologic, and toxicology findings to properly identify cause and manner of death as it relates to the continued emergence of novel opioid use and abuse.

POSTER

P-064: New Psychoactive Substance (NPS) identification by analyses of oral fluid samples collected at electronic music festivals in Brazil

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Background/Introduction: New psychoactive substances (NPS) are a worldwide problem and many countries have tried to monitor the NPS occurrence in their territories. In Brazil, there is no official information available regarding NPS consumption. Research involving NPS in international communities is important for comparison on the world stage, as many of these substances are later seen in other countries.

Objectives: This work aimed to evaluate the prevalence of NPS in Brazil using oral fluid samples collected at 2 electronic music festivals with analysis by both liquid chromatography triple quadrupole mass spectrometry (LC-MS/MS) and liquid chromatography high-resolution mass spectrometry (LC-HRMS).

Methods: Oral fluid samples were collected using the Quantisal™ device (Immunalysis, Pomona, CA). Volunteers were >18 years old and had used an illicit psychoactive substance in the last 24 h preceding sample collection. Oral fluid samples from both festivals were separated in two aliquots. One of them were used to perform a liquid-liquid extraction using 500 μL sample and 2 mL tert-butyl methyl ether. The reconstituted organic phase was injected into an LCMS8060 (Shimadzu, Kyoto, Japan). Second aliquot of the samples were shipped to the Center for Forensic Science Research and Education (PA, USA) for further investigation. A liquid-liquid extraction was performed using 500 μL sample and 3 mL n-butyl chloride:ethyl acetate (1:1, v/v). The reconstituted organic phase was injected into TripleTOF 5600+ (Sciex, Ontario, Canada).

Results: 230 oral fluid samples were collected during electronic music festivals that occurred in two Brazilian states in winter 2018 and summer 2020. Eleven different NPS were identified in these samples by LC-MS/MS. Ketamine was the most prevalent NPS (46%), followed by the synthetic cathinones methylone, N-ethylpentylone, dipentylone and eutylone. Two additional NPS were identified by LC-HRMS: 4-CDMC and 25E-NBOH. MDMA was the most prevalent (88%) drug of abuse identified within samples, followed by THC (83%), MDA (69%) and LSD (30%). Overall, there were 29 different drugs, including conventional and NPS, identified within these samples. Poly-drug consumption was observed in 88% of the samples, mostly for 2-4 substances.

Table 1. NPS identified (% of total) in 230 oral fluid samples.

LC-MS/MS		LC-HRMS	
ketamine	106 (46.1)	ketamine	83 (36.1)
methylone	15 (6.5)	methylone	3 (1.3)
N-ethylpentylone	11 (4.8)	N-ethylpentylone	2 (0.9)
dipentylone	6 (2.6)	eutylone	3 (1.3)
eutylone	4 (1.7)	4-CDMC	1 (0.4)
25I-NBOH	4 (1.7)	25E-NBOH	1 (0.4)
5-MeO-MiPT	3 (1.3)		
25C-NBOH	1 (0.4)		
4-CEC	1 (0.4)		
mCPP	1 (0.4)		
MDPV	1 (0.4)		

Conclusion/Discussion: This paired mass spectrometry approach was successful for detecting NPS in oral fluid samples. About 60% of NPS findings by LC-MS/MS were also discovered using LC-HRMS; the discrepancies could be associated with long sample storage times between the first and second analysis (33 and 14 months, respectively) and/or the sensitivity differences of each assay. The limits of detection for 5 analytes (ketamine, eutylone, N-ethylpentylone, methylone, 4-CEC) were between 0.05-0.1 ng/mL by LC-MS/MS and 0.75-10 ng/mL by LC-HRMS. LC-MS/MS requires added work to keep an updated library and samples must be re-analyzed to obtain past data; LC-HRMS is often easier to expand the scope of testing and data-mining alleviates the need for re-analysis. The high rates of poly-drug consumption observed in these events are a concern and can increase intoxication and toxicity risks. This is the first work to bring information on NPS consumption in Brazil. Oral fluid sampling proved to be an effective tool for onsite collection and NPS monitoring.

POSTER

P-065: Cannabidiol in Acidic Foods

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Background/Introduction: The 2018 Agriculture Improvement Act (Farm Bill) removed certain hemp-derived products from the Controlled Substances Act (CSA) list. This Act has led to the proliferation of hemp-derived cannabidiol (CBD) products (e.g., tinctures, oils, creams), often marketed as remedies for a range of medical conditions. Although, the U.S Food and Drug Administration (FDA) has approved the usage of CBD in the form of Epidiolex®, currently there is no regulatory oversight by the Federal Government to ensure the accuracy of label information and marketing claims for "over-the-counter" products containing CBD. Availability of such products pose many questions for workplace drug testing including, whether or not these products contain sufficient Δ9-tetrahydrocannabinol (THC) to cause a positive urine test and whether the acidic environment of some food products may cause CBD to be converted to THC [1,2]. Investigation into these possibilities must include measurement of cannabinoids in CBD marketed products, controlled dosing studies and laboratory studies of CBD in acidic environments.

Objectives: The primary objective of this preliminary study was to quantitate CBD, THC and minor cannabinoid content in a variety of food and beverage products advertised by manufacturers as containing CBD and likely to have a pH of <7.

Methods: Products were selected for purchase in person at national retailers and online specialty websites. Analysis of products was performed by ElSohly Laboratories, Incorporated and included quantitative analysis for Δ9-THC and CBD, their acid precursors, and semi-quantitative analysis for 5 minor cannabinoids with LOD of 0.1 μg/mL(g) using previously published methods[3,4]. Other product parameters collected included price, type of product, total amount of THC and CBD in the product and amount per serving (calculated). Results were compared to labeling or other information offered by the manufacturer.

Results: Forty-seven products were selected for purchase and analysis, which included beverages, condiments, chocolates, gummies, and snacks. Prices ranged from $2.39 USD for a single item (Hemp milk, 32 oz) to $78 USD for a 12-pack of Kombucha. Twenty-nine of the 47 (61.7%) products included a reference to FDA having not evaluated the product or its claims or similar warning while 18 of 47 (38.3%) made no mention of the FDA. Product labeling for CBD content was inconsistent and stated that the range of total CBD for products was 10-500 mg per container/serving. However, analytical testing of CBD ranged from 0 to 3700 mg per container/serving. Only four of the products had CBD results within 10% of product labeling. The acid precursor of CBD was detected in 4 products, THC was detected in four products (0.1, 8, 20, and 117 mg) and the minor cannabinoids CBC and CBG were detected in two products. The acid precursor of THC was not detected in any product. Six of the products contained no CBD (< LOD) while others from the same manufacturer contained the amount claimed.

Conclusion/Discussion: Preliminary findings on CBD/THC in acidic foods and beverages indicate that there is wide variation in label consistency with product claims for CBD content and actual results. Further studies are planned to examine the effect of product packaging, storage and food acidity on CBD stability.

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POSTER

P-066: Reaching New Sensitivity Levels for the Detection of Fentanyl Analogs and Highly Potent Novel Synthetic Opioids in Blood

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Background/Introduction: The continuous emergence of novel synthetic opioids (NSO) on the recreational drug market has been a major contributor to the ongoing opioid crisis. NSO are a class of novel psychoactive substances (NPS) that includes analogs of fentanyl and newly emerging non-fentanyl compounds. As newer and more potent synthetic opioids are synthesized and introduced to the recreational drug market, timely and comprehensive analytical drug screening approaches focused on rapid identification of these novel substances in biological matrices are critically needed.

Objectives: The objective of this study was to develop a quantitative workflow for the analysis of 32 of potent substances in poly-drug, authentic case samples at low (pg/mL) levels. The method provides a fast analytical method that provides toxicologists a better picture of the overdose causation.

Methods: A total of 32 NSO including 17 fentanyl analogs and 15 newly emerging non-fentanyl opioids were selected for this panel. NSO were extracted from the 200 µL of spiked whole blood mixtures using a liquid-liquid extraction (LLE) procedure. Analytes were chromatographically separated using a Phenomenex Kinetex C18 column (50 × 3.0 mm, 2.6µm, 00B-4462-Y0). Mobile phase A (MPA) and mobile phase B (MPB) were ammonium formate with formic acid and formic acid in methanol and acetonitrile, respectively. The injection volume was 10 µL and the total LC runtime was 15.5 minutes. A single acquisition method consisting of 68 MRM transitions (64 for the NSO and 4 for the internal standards) was created using the Scheduled MRM Algorithm.

Results: Control human whole blood samples spiked with the 32 target analytes were prepared at concentrations ranging from 1 pg/mL to 100 ng/mL. Excellent linearity was observed across the concentration ranges analyzed with R^2 values greater than 0.99 for all of the NPS in the panel. In addition. reported LLOQ values ranged between 10 to 50 pg/mL for the 32 analytes in the panel. The accuracy and precision of measurements ranged from 80.50-116.64% and from 0.42-17.80%, respectively. The excellent accuracy and precision were observed over the entire concentration range, including at the LLOQ. Overall, the developed method showed excellent reproducibility and linearity, proving the robustness of the developed method and quantitative performance of the SCIEX 7500 System even at low concentration levels for each of the targeted NSO in this study. The applicability of the method to analyze discarded authentic postmortem case samples from subjects suspected of NPS ingestion resulting in accidental overdoses was investigated. For example, the results from the analysis of one of these case samples showed the successful detection of one NSO and its metabolite: isotonitazene and 5-aminoisotonitazene, as well as fentanyl and four of its analogs/metabolites: beta-hydroxy-fentanyl, norfentanyl, 4-ANPP and acetyl fentanyl at concentrations of 1434.33, 7.93, 599.10, 9756.67, 147.69 and 1465.00 pg/mL, respectively. The results demonstrate that the developed method enabled accurate detection of potent substances in poly-drug, authentic case samples at trace levels that were not previously achievable.

Conclusion/Discussion: A robust and sensitive drug screening workflow for the analysis of 32 potent NSO was successfully developed using the SCIEX 7500 System. Overall, the remarkable quantification performance of the SCIEX 7500 System enabled accurate detection of potent NSO at concentrations that were not previously achievable, providing a means to monitor ultra-potent NSO in overdose scenarios. The sensitivity levels afforded by the SCIEX 7500 system provided the ability to detect low levels of NSO in postmortem case samples that would normally go undetected, providing a clearer picture for help in determining the cause of death.

POSTER

P-067: Development of a UPLC-MSMS method for the identification of 52 Fentanyl analogs and 7 isotopically labelled internal standards

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Background/Introduction: In the past 14 years, there has been an increase of reported opioid related overdoses in the United States. This increase is even more pronounced with the current pandemic. According to Centers for Disease Control and Prevention (CDC), heroin overdoses tripled from 2010 to 2013. While this increase occurred heroin overdoses have remained steady, since 2016, there has been a steeper increase in overdose deaths attributed to synthetic opioids. In the US in 2018, over 67,000 drug related overdose deaths were recorded. The main cause was predominantly synthetic opioids (other than methadone). With opioids accounting for or being involved in almost 70% of all drug overdose deaths, and over two thirds involved synthetic opioids. These increases correlate with drug secures and testing from law enforcement agencies, rather than actual increases in legal prescriptions. Therefore, the increase is proposed to be from illicit manufacturing and distribution other than legally obtained prescriptions and dispensing pharmaceuticals. These synthetic opioids commonly contain fentanyl analogs and other chemically similar compounds as results of their non-regulated nature. With varying amounts present and varying potency of these analogs, it is difficult to determine the potential toxicity of the mixtures. Since 2018, the US Centers for Disease Control and Prevention (CDC) has made available the Fentanyl Analog Screening Kit and Emergent Panels (TOM Kits), which contain previously unavailable fentanyl analog reference materials. There are several published manuscript containing methods to analyze for single or limited number of analogs, but few address the analysis of isomeric analogs in a single class let alone multiple classes and/or isobars.

Objectives: Develop a screening method for the separation and identification of 52 fentanyl analogs and 7 isotopically labelled internal standards using an ultra-high performance liquid chromatography (UPLC-MSMS) using Multiple Reaction Monitoring (MRM).

Methods: The 52 fentanyl analogs (fentanyl, norfentanyl, and fentanyl analog classes: acryl, butyryl, furanyl, and thio), and 7 of their isotopically labeled internal standards were infused on a Waters AcQuity Xevo TQS-micro Ultra performance liquid chromatography - tandem mass spectrometer (UPLC-MS/MS) system with a electrospray ionization (ESI) in positive ion mode. The appropriate transition ions (m/z) and collection energies (CEs) were determined for each analog in multiple reaction monitoring (MRM) mode. The chromatographic resolution was evaluated on six column different stationary phases from four different manufactures using various mobile phase compositions and gradients.

Results: The best chromatographic resolution of the 59 compounds was achieved using either a Waters ACQUITY UPLC BEH C18 (1.7um, 2.1mm x 100mm) or a Waters XSelect HPLC CSH C18 XP (2.5um, 3mm x 100mm) column. The optimum mobile phase and chromatographic gradient was obtained using (A) 0.1% formic acid in water and (B) 0.1% formic acid in acetonitrile. The gradient elution conditions were an initial 1-minute hold at 15% B, 16-minute Curve 7 gradient to 60% B, 0.8-minute ramp to 95%B and hold for 0.7-minutes, 0.5-minute ramp to 15% B and hold for 1.5-minutes. This resulted in separation and identification of 46 of the 59 analogs on both the BEH and CSH columns. Additionally eight isobars were chromatographically separated using a Phenomenex Biphenyl (1.7um, 3.2mm x 100mm) column with the same chromatographic conditions. Six butyryl analogues were identified as 2 distinct groups but were not chromatographically separated; o,m,p-fluoro furanyl fentanyl, and o-fluoro isobutyryl fentanyl and m,p-fluoro butyryl fentanyl.

Conclusion/Discussion: The UPLC-MS/MS methods were able to chromatographically resolve the isobaric acryl and thio fentanyl analogs. With the additional biphenyl column, all isobars were able to be chromatographically resolved except one set of furanyl fentanyl isobars and one set of butyryl fentanyl analogs.

POSTER

P-068: Rapid Identification and Quantification of Stimulants in Human Urine Using High-Resolution Mass Spectrometry

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Background/Introduction: Stimulants are a diverse group of substances that include both prescription medications and illegal street drugs, as well as commonly used legal substances such as caffeine and nicotine. As the misuse of these drugs continues to increase rapid, robust and comprehensive detection methods that allow positive identification and accurate quantification of stimulants in biological samples are critically needed.

Objectives: The objective of this study is to develop a comprehensive workflow combining the use of the SCIEX X500R QTOF System with a fast sample preparation procedure for the sensitive detection of a structurally-diverse panel of stimulants in human urine. The method enables robust and reproducible detection of a panel of 15 stimulants in human urine with ng/mL detection limits.

Methods: A 10 µg/mL stock standard mixture, containing the 15 stimulants targeted in this study, was prepared by diluting stock standards with methanol. The stimulants included in the panel are cathine, cathinone, amphetamine, cocaine, phendimetrazine, fenethylline, fenproporex, phentermine, mephentermine, methamphetamine, methylhexanamine, methylphenidate, pemoline, strychnine, and benzphetamine. Blank human urine samples were fortified with the stock standard mixture at various concentration levels. Fortified urine samples were diluted 10-fold with a solution of acetonitrile/methanol (80/20, v/v) and subsequent dilutions were performed to give desired concentrations ranging from 100 to 1000 ng/mL. The resulting samples were centrifuged, and the organic phase was transferred to a sample vial for analysis. Analytes were chromatographically separated at 45˚C using a Phenomenex Kinetex C18 column (100 x 2.1 mm, 1.7 µm, 00D-4475-AN). Mobile phases used consisted of HPLC grade water with 0.1% formic acid (mobile phase A) and acetonitrile with 0.1% formic acid (mobile phase B). The LC flow rate was 0.5 mL/min and the total run time was 7 min. The injection volume was 1 µL. MS and MS/MS data were collected for each sample using SWATH Acquisition on the SCIEX X500R QTOF System in positive mode. Data acquisition was TOF MS scan followed by 12 MS/MS scans using variably sized Q1 windows covering a mass range from 100 to 350 m/z. The resulting cycle time was 0.555 sec.

Results: The quantitative performance of the assay was investigated by injecting a series of six calibrator solutions prepared in urine. Calibration curves were generated to evaluate the response and quantitation performance for each of the 15 stimulants used in this study. The calibration curves showed excellent correlation and linearity with R^2 values >0.99 for all the stimulants in the panel. In addition to determining linearity and correlation, the following validation parameters were calculated for the 15 stimulants in the panel: LODs, LOQs, inter- and intra-assay precision and accuracy as well as matrix effect. Limits of quantitation (LOQ) and detection (LOD) for the 15 stimulants in matrix ranged from 3.3 to 100 ng/mL and 1.0 to 30 ng/mL, respectively. Matrix effects ranged between ±2.2 and ±35.7% for all 15 analytes, suggesting no significant ion suppression or enhancement was observed for any of the stimulants in the panel. Lastly, intra-day precision and accuracy were calculated for the detection of the 15 stimulants in urine samples. The values were found to be below 20% for both the CV% and the bias%, for the calibrators at 100, 500 and 1000 ng/mL concentration.

Conclusion/Discussion: A comprehensive workflow for the detection of stimulants in human urine was successfully developed using the SCIEX X500R System. A rapid sample preparation procedure in combination with a highly selective MS/MS method with SWATH Acquisition enabled robust and reproducible detection of a panel of 15 stimulants in human urine with ng/mL detection limits.

POSTER

P-069: Identification and Quantitation of Fluorofentanyl in Postmortem Blood

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Background/Introduction: The opioid epidemic continues to evolve in the United States with fentanyl being most prevalent. Following the scheduling of fentanyl's core structure in 2018, there has been a notable decline in the prevalence of fentanyl analogs in decedents. Fluorofentanyl has three positional isomers (*para*-fluorofentanyl, *ortho*-fluorofentanyl, and *meta*-fluorofentanyl) with the most prevalent isomer that has recently emerged in the United States being *para*-fluorofentanyl. It has been reported that *para*-fluorofentanyl possesses similar potency to fentanyl.

Objectives: The goal of this study was to identify *para*-fluorofentanyl in postmortem cases between the end of 2020 and beginning of 2021.

Methods: *LC-MS Analysis:* Urine (500 μL) was extracted using UCT Clean Screen® Extraction Columns. Internal standard (fentanyl-d5, 50 μL) was added to the matrix and buffered with 2 mL of pH 6 phosphate buffer (0.1 M) then centrifuged. The extraction columns were conditioned with 3 mL of methanol, deionized water, and phosphate buffer. After conditioning, samples were added to the columns and washed with 3 mL water and 1 mL of 1 M acetic acid. The columns were dried under positive pressure then washed with organic (hexane and methanol) and eluted with methylene chloride:isopropanol:ammonium hydroxide (78:20:2, v/v/v). Samples were dried down under nitrogen and reconstituted in 200 μL mobile phase. The screen was performed using an Agilent 1290 Infinity Liquid Chromatograph coupled to an Agilent 6545 Accurate Mass Time-of-Flight Mass Spectrometer. Chromatographic separation was achieved using an Agilent Poroshell 120 EC-C18 (3.0 x 100 mm, 2.7 μm) column coupled with matching guard column. The limit of detection was 0.1 ng/mL. The method could not differentiate the three positional isomers of fluorofentanyl. *GC-MS Analysis:* Blood/urine (1 mL) was extracted using the SPE as described above and analyzed by an Agilent 6890N GC system coupled with an Agilent 5973 MS. Samples were reconstituted in 50 μL of methanol. Chromatographic separation was achieved using a Zebron ZB-5MSplus (30 m x 0.25 mm, 0.25 μm) column. The linear range for this method was 2.5-50 ng/mL (limit of detection: 0.63 ng/mL) for fentanyl, acetyl fentanyl, butyryl fentanyl, *para*-fluorofentanyl, *ortho*-fluorofentanyl, and *meta*-fluorofentanyl.

Results: Cases (n=270) received from October 2020 through March 2021 from four Medical Examiner Districts in the state of Florida (Gainesville, Winter Haven, Sarasota and Marathon) were analyzed for the presence of fluorofentanyl. The LC-QTOF-MS screen yielded 19 decedents positive for fluorofentanyl. The majority of these decedents were Caucasian (95%) and male (84%) with ages ranging from 27 to 54 years old (median: 36 years old). Analysis of the blood and urine by GC-MS yielded ten decedents positive for *para*-fluorofentanyl, eight of which were positive in the blood. Fentanyl was detected in all of the *para*-fluorofentanyl positive decedents. Blood concentrations are indicated below:

Case	Age	Fentanyl (ng/mL)	*para*-Fluorofentanyl (ng/mL)
1	29	11	<LOQ
2	49	26	14
3	50	49	30
4	40	17	9
5	38	10	4.1
6	32	3.0	3.9
7	34	32	<LOQ
8	27	26	2.5

Conclusion/Discussion: LC-QTOF-MS was successfully used to screen urine for the presence of fluorofentanyl; however, the method was unable to differentiate the isomers of fluorofentanyl. In order to ensure the definitive identification of the isomer of fluorofentanyl, the blood and urine were subjected to GC-MS analysis. Fluorofentanyl was identified in the blood of 42% of the cases, and the concentration of *para*-fluorofentanyl was generally higher than previously reported. The prevalence of *para*-fluorofentanyl will continue to be monitored.

POSTER

P-070: Comparison of Postmortem Toxicology with Seized Drug Evidence from Death Scenes

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Background/Introduction: Drug Delivery Resulting in Death (DDRD) charges have increased over 365% in the state of PA over the last 5 years[1]. Successful prosecution is determined by many factors, not the least of which is association between postmortem toxicology findings and analysis of seized drug evidence from the death scene and those confiscated from suspect dealers. Although the relationship between analyzed drug evidence and the toxicological profile of a decedent is, at times, descriptive, a strong methodological and statistical analysis of the relationship of compounds observed in both analyses could be used to facilitate prosecutions.

Objectives: This work will compare five grouped cases where seized drug evidence was obtained at the death scene of overdoses. A statistical analysis of related chemical components between the two data sets will be performed and used to elucidate relationships between postmortem blood levels and drug evidence. This project can provide a framework to further develop models for studying interdependent forensic cases.

Methods: Blood toxicology specimens were extracted by phospholipid depletion and protein precipitation. The extracts were analyzed by LC-MS/MS methods using HRAM and QqQ screening followed by SRM quantitative confirmations. Broad scope screening by UPLC-MS/MS was accomplished on a Phenomenex Biphenyl column (2.1 x 50mm, 2.6 um) with 10 mM ammonium formate, pH 4.0 and acetonitrile. Multiple quantitative, confirmation methods were utilized dependent on analytes screened, separated on a Phenomenex C18 column (2.1 x 100 mm, 2.6 um) with 10 mM ammonium formate pH 4.0 and acetonitrile. Additionally, seized drug evidence collected at the death scene from these cases were analyzed using a combination of colorimetric screening (Marquis) and GC-MS to identify compounds in the drug evidence. Seized evidence testing involved extraction with methanol and analysis on a Restek Zebron ZB-1 (30 m x 0.25 mm ID, 0.1 μm) with a temperature ramp to 330°C.

Results: In five paired cases where seized drug scene evidence was analyzed along with postmortem whole blood specimens, three cases demonstrated the presence of typical compounds fentanyl and heroin metabolites. However, in two separate cases, additional compounds were observed in both the scene evidence as well as quantifiable levels in the blood specimens. These "fingerprint" compounds were 4-ANPP, xylazine, and lidocaine in case 4, while MDMA, MDA, tramadol, p-Fluorofentanyl, lidocaine as well as other agents were observed in case 5. Additional and current cases will continue to be evaluated.

Conclusion/Discussion: Five paired cases were analyzed for their scene drug evidence and postmortem blood levels. The goal of these analyses is to facilitate or strengthen the prosecution of defendants charged with DDRD, through the association of multiple chemical compounds in both seized drug and postmortem findings. Unique challenges are present when seized drug evidence is highly pure or only contains a few commonly found compounds, however as the drug mixtures become more complex, highly sensitive toxicology methods can reveal several "fingerprints", strengthening the association.

POSTER

P-071: Fentanyl and Methamphetamine: The New Speedball in Alabama

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Background/Introduction: The street term "Speedball" has historically referred to a combination of heroin and cocaine. This combination has famously led to the overdose deaths of comedians John Belushi and Chris Farley, as well as actors River Phoenix and Phillip Seymour Hoffman. The danger of this combination lies in the increased risk of respiratory failure: the stimulant causes your body to use more oxygen, while the opioid slows your respiratory rate. Some users also report a "canceling-out effect", which may lead to a false sense of sobriety, causing users to take additional drugs and overdosing. While heroin and cocaine are the classical components of a "Speedball", trends in illicit drug use have shifted in recent years and vary by location. For example, the prevalence of fentanyl in opioid overdoses has significantly increased in the United States. In Alabama, methamphetamine is often more common than cocaine. Both drugs are largely produced in Mexico and transported into the US, including Alabama. Though fentanyl and methamphetamine have been seen in casework in other parts of the country over several decades, this is a new trend in Alabama. Sale of these drugs has only increased due to the COVID-19 pandemic; methamphetamine popularity in particular has grown due to low costs despite increased purity. The surge of cases with these two drugs has led to an increased incidence of the drugs being found together.

Objectives: To investigate the prevalence of methamphetamine and fentanyl in cases analyzed by the ADFS between 2016 and 2020, to evaluate their concentration in blood samples and to identify user demographics.

Methods: Death cases and DUI/D cases were screened by ELISA using the Tecan Evo75 with Immunalysis reagents or the Randox Evidence Analyzer. Confirmation of methamphetamine in blood was performed by solid-phase extraction using Clean Screen SPE with GC/MS. Confirmation for fentanyl in blood was performed by liquid-liquid extraction with LC-MS/MS. Microsoft Excel was used to filter and sort the data.

Results: From 2016-2020, the ADFS reported 924 fentanyl results and 2,223 methamphetamine results for DUI/D and overdose cases. Of these cases, 53 DUI/D cases and 201 overdose cases with both methamphetamine and fentanyl present were identified. Demographics for the 53 DUI/D cases were: 75% men, 25% women; 92% white, 6% black, and 2% Asian. Demographics for the 201 overdose cases were: 70% men, 30% women; 90% white, 9% black, 1% Asian. The average age in DUI/D cases was 35, while the average age in overdose cases was 40. The median concentration in DUI/D cases was 240 ng/mL for methamphetamine and 2.3 ng/mL for fentanyl. Of the overdose cases in our reportable calibration range, the median concentration was 250 ng/mL for methamphetamine and 10 ng/mL for fentanyl.

Conclusion/Discussion: In 2020, presumptive positive cases for methamphetamine and fentanyl increased. Based on the data trends for the last five years, the combination of methamphetamine and fentanyl has increased in prevalence in Alabama. There was no relevant increase in combination DUI/D cases for this time period, and for the four years prior to 2020, there was also no marked increase in overdose combination cases; however, the number of overdose cases increased from 39 in 2019 to 108 in 2020, a 276% increase in cases. This shows that the combination of these two drugs has become quite popular in Alabama over the last year. This could be a result of the increased sale of illicit drugs due to the COVID-19 pandemic. Over 28% of the methamphetamine results exceeded the upper limit of quantitation (1000 ng/mL), and over 10% of the fentanyl results exceeded the upper limit of quantitation (30 ng/mL), demonstrating the tolerance of some users for these drugs at high doses.

P-072: Buprenorphine co-positivity with designer benzodiazepines in routine healthcare urine samples

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Background/Introduction: Novel psychoactive substances (NPS) continuously evolve, with observation of designer benzodiazepine (DBZD) discussions in online drug forums, increased publications in the scientific literature, and increased identifications in forensic samples. DBZD are used as part of "upper downer cycles" to achieve sedation and/or amnesia, or to potentiate the effects of opioids or prescription benzodiazepines. The combination of prescription benzodiazepines and buprenorphine requires careful monitoring to minimize the risk of accidental injury and resultant increase in healthcare utilization, especially emergency department visits. Due to their unknown potency, DBZD may be more, or less, harmful than their prescription counterparts, and identification of these compounds can better inform healthcare providers of their patients' substance use history.

Objectives:

1. Investigate the rate of buprenorphine detection in DBZD-positive samples.
2. Evaluate the positivity rate of specific designer benzodiazepines in urinary specimens.
3. Assess the need for metabolite testing when developing a testing method for designer benzodiazepines.

Methods: This study was IRB approved. DBZD were analyzed in the laboratory as part of a larger NPS panel. Target analytes were included in the method based on information from the US Drug Enforcement Administration and other online resources. Prior to analysis, analytes were extracted from hydrolyzed urine using a liquid-liquid extraction followed by evaporation and reconstitution in mobile phase. Samples were injected onto a liquid chromatography/tandem mass spectrometry (LC-MS/MS) instrument consisting of a Shimadzu Prominence HPLC and Sciex API 4000 MS/MS. The mass spectrometer was operated in positive electrospray ionization mode for scheduled multireaction monitoring (sMRM) analysis. Analytes were chromatographically separated on a Biphenyl column. Upon request by a provider during the study period of January 2021, 3,892 samples were analyzed for bromazolam, clonazolam, 8-amino-clonazolam, diclazepam, delorazepam, etizolam, alpha-hydroxyetizolam, flualprazolam, alpha-hydroxyflualprazolam, flubromazepam, flubromazolam, alpha-hydroxyflubromazolam, nitrazolam, phenazepam, or 3-hydroxyphenazepam, with reporting thresholds ranging from 1-5 ng/mL. Buprenorphine was analyzed separately in hydrolyzed urine, with reporting thresholds of 1 ng/mL for buprenorphine and 2.5 ng/mL for norbuprenorphine. A positive result in all instances included samples where the parent and/or metabolite were detected above the threshold.

Results:

Overall Sample Characteristics	Specimen Tested, No (%)
Number of samples with DBZD test ordered	3,892
Number of DBZD-positive specimens	30 (0.7)
Median patient age of DBZD-positive specimens	39
Male	14/30 (46.6)
Female	16/30 (53.4)
Buprenorphine Positive Result	**17/30 (56.6)**
Designer Benzodiazepine Present with Buprenorphine:	
Alpha-hydroxyetizolam[a]	7/17 (41.1)
8-aminoclonazolam[a]	9/17 (52.9)
Flualprazolam	5/17 (29.4)
Alpha-hydroxyflualprazolam[a]	9/17 (52.9)
Bromazolam	1/17 (5.8)

[a] Metabolite

Conclusion/Discussion: Of 3,892 tests requested, 30 (0.7%) tested positive for at least one DBZD, with eight samples containing greater than one. Of the 30 samples, 17 were also positive for buprenorphine. Importantly, testing for known metabolites improves detection efficiency. Of the 30 DBZD-positive specimens, metabolites were detected in the absence of parent drug in 26 of 30 samples (86.6%). This study demonstrates that individuals seeking routine healthcare may test positive for DBZD during office visits and providers may benefit from this knowledge to allow for close monitoring for adverse events from interacting substances. Per US Food and Drug Administration guidance, one known interacting drug with prescription benzodiazepines is buprenorphine, which we detected in 56.6% of the 30 DBZD-positive samples. Through innovative analytical techniques and education of healthcare providers, the identification of DBZD may potentially play a role in the reduction of adverse events associated with the combination of buprenorphine and benzodiazepines and facilitate the safer use of buprenorphine for individuals with opioid use disorder.

POSTER

P-073: The Effect of Designer Benzodiazepines on ELISA Confirmation Rates

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Background/Introduction: Enzyme-linked immunosorbent assay (ELISA) is a standard method of screening biological samples for drugs. In ELISA testing, benzodiazepine kits target traditional benzodiazepines, such as oxazepam, but are variably reactive with designer benzodiazepines. Since their first detection in 2017, the Harris County Institute of Forensic Sciences (HCIFS) has not systematically studied the cross-reactivity of designer benzodiazepines on our ELISA method or the impact that designer benzodiazepines have on confirmation rate.

Objectives: This research aims to determine the cross-reactivity of designer benzodiazepines on ELISA and casework confirmation rates from January 2017 to March 2021. This research also aims to identify which designer benzodiazepines are being missed by our confirmation analysis.

Methods: The cross-reactivity of 16 designer benzodiazepines (alpha-hydroxyetizolam, delorazepam, deschloro-etizolam, flualprazolam, flubromazepam, flubromazolam, adinazolam, nitrazolam, bromazolam, flunitrazepam, flunitrazolam, meclonazepam, N-desmethyl flunitrazepam, 8-Aminoclonazolam, bromazepam, and pyrazolam) was assessed by running 20 ng/mL samples in triplicate in blood and urine using the HCIFS ELISA procedure. Cross-reactivity was calculated as the absorbance ratio of analyte to oxazepam target, multiplied by 100. Benzodiazepine ELISA and confirmation results for cases from January 2017 to March 2021 were extracted and the confirmation rate was calculated. Confirmation testing for 16 benzodiazepines was performed by liquid chromatography tandem mass spectrometry with limits of detection of 0.5-5 ng/mL. The novel benzodiazepines in this method are clobazam, etizolam, flubromazepam, midazolam, and phenazepam. To see which designer benzodiazepine(s) were missed during confirmation testing, time of flight (TOF) data, where available, were retrospectively mined for designer benzodiazepines using molecular weight searches; additionally, where applicable, designer benzodiazepine testing performed by a reference laboratory was used to determine what designer benzodiazepines were being missed.

Results: The cross-reactivity study showed that alpha-hydroxyetizolam, delorazepam, deschloroetizolam, flualprazolam, flubromazolam, flubromazolam, adinazolam, nitrazolam, bromazolam, flunitrazepam and flunitrazolam are 100-184% cross-reactive in blood and 96-216% in urine. Meclonazepam and N-desmethyl flunitrazepam had poor cross reactivity in blood and urine; their cross reactivity was 68% and 89%, respectively, in blood and 65% and 79% in urine. 8-Aminoclonazolam, bromazepam, and pyrazolam had variable cross reactivity across the matrices. Their cross reactivity was 83-87% in blood and 94-123% in urine. From January 2017-March 2021, the benzodiazepine confirmation rate was 83%. The number of confirmation tests performed increased each year, but the confirmation rate decreased from 95% in 2017 to 75% in 2021. Medicolegal (ML) death investigation cases had a confirmation rate of 84%, while driving under the influence and drug facilitated sexual assault cases (IFS) had a confirmation rate of 82%. From 2017 to 2021, the confirmation rate for ML cases decreased from 95% to 82%. For IFS cases, it decreased from 94% to 67%. Forty-one cases were tested either by TOF screen or a reference laboratory: flualprazolam (n=9), flubromazolam (n=2), flunitrazolam (n=7), nimetazepam (n=1), and alpha-hydroxytriazolam (n=1) were identified as designer benzodiazepines that have been missed at confirmation. In four cases, flualprazolam was identified along with either flunitrazolam (n=2), alpha-hydroxytriazolam (n=1), or alpha-hydroxyetizolam (n=1).

Conclusion/Discussion: Over the last four years, the proliferation of designer benzodiazepines has shown the importance of up-to-date methods to maximize drug identification and confirmations rates. This study showed that the majority of designer benzodiazepines are capable of being detected on ELISA at 20 ng/mL; however, some benzodiazepines, such as flualprazolam, are observed in lower concentrations in casework. A secondary screening technique with higher sensitivity and selectivity, such as TOF, may be required. With the combination of the cross reactivity on ELISA and a TOF screen and confirmation method with the addition of the novel benzodiazepines, HCIFS will able to improve upon their 83% confirmation rate.

Key Words: ELISA, Designer, Benzodiazepines, Confirmation Rate

POSTER

P-074: Analysis of Psilocin and Psilocybin, in Urine Using High-throughput LC-MS/MS

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Background/Introduction: Recently, a few counties and municipalities in the US have decriminalized small doses, or micro-doses, of the psychedelic drugs psilocin and psilocybin. These two hallucinogenic indole alkaloids are commonly found in the street drug "magic mushrooms". Due to the increased interest in these compounds, a simple and robust analytical method is needed for monitoring these compounds in biological matrices. These compounds can be problematic to analyze as they are highly polar in nature and can suffer from matrix interference. Additionally, psilocybin can break down to psilocin by cleaving the phosphate moiety due to the in-source fragmentation via Liquid chromatography tandem mass spectrometer requiring baseline separation. In this study, we developed a robust and fast LC-MS/MS method for the separation and quantitation of psilocin and psilocybin in urine employing a simple solvent precipitation.

Objectives: The intent of this study was to develop and partially validate a high throughput LC-MS/MS method for the separation and quantitation of psilocybin and its metabolite psilocin in urine, using a reversed-phase column following protein precipitation and dilution with a total run time of 3.5 minutes.

Methods: Ten individual lots of human urine (BioreclamationIVT) was screened for both psilocin and psilocybin and pooled to prepare calibration and Quality Control standards. The linearity ranges were from 50-5,000 ng/mL for both analytes, with four QC levels prepared at 50, 125, 700 and 4000 ng/mL. The samples were prepared using a simple solvent precipitation method – a 50 μL sample aliquot is taken, mixed with internal standard and methanol, then vortexed and centrifuged. After centrifugation, the supernatant was diluted with mobile phase A and injected on a Raptor Biphenyl column (50 x2.1mm, 2.7 μm) with an EXP guard column using a robust, reversed-phased mode. A fast cycle time of 3.5 minutes was achieved, including column re-equilibration, that resulted in full resolution of the target compounds, with no carryover observed, on a Shimadzu Prominence HPLC coupled with a SCIEX API 4000™ using electrospray ionization in positive ion mode.

Results: Psilocin and psilocybin were successfully analyzed in urine. Partial validation across a linear range of 50-5000 ng/mL demonstrated good linearity with r^2 values of 0.995 or greater. Acceptable method accuracy and precision was demonstrated across multiple days with %Relative standard deviation <18% and percent recovery within 15 % of the nominal concentrations for low, mid, and high QC and LLOQ levels, respectively.

Conclusion/Discussion: A simple solvent precipitation and fast LC-MS/MS quantitative analysis for psilocybin and psilocin in human urine was successfully developed. The use of this analysis should greatly assist the labs to not only detect and quantify both psilocin and psilocybin in urine, but also easily add these compounds to their existing drugs of abuse/pain management panel.

POSTER

P-075: Analysis of fatalities involving amphetamine in Jazan, Saudi Arabia

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Background/Introduction: Amphetamine abuse has become a major concern facing the people of Saudi Arabia. It is frequently abused for its euphoric and stimulant effects. Overdose or chronic excessive use results in tachycardia, hypertension, agitation, and psychosis. Amphetamine is associated with high tendencies for homicidal or suicidal deaths. The analysis of fatalities involving amphetamines is necessary to develop policies for preventative purposes.

Objectives: This study analyzed fatalities involving amphetamine in Jazan from 2018–2020 and the postmortem tissue distribution of amphetamine was investigated

Methods: All fatal cases reported to Poison Control & Medical Forensic Chemistry Center between January 2018 to December 2020 was to evaluate retrospectively by using consecutive sampling. The manner of death is classified as following: suicidal, homicidal, accidental, and undetermined. The toxicological analysis results and quantification results were collected and investigated.

Results: In comparison to all fatalities involving amphetamine, the number of fatalities involving amphetamine with other drugs has proportionately increased from 18% in 2018 to 55% in 2019 and to 80% in 2020. The highest average blood concentrations (2.32 mg/L) were in suicidal, with highest 90th percentile blood concentrations (7.6 mg/L). The number of suicidal and homicidal deaths in amphetamine with other drugs group are higher than in amphetamine alone group. 78% of all amphetamine fatalities involved ethanol, THC, and cathinone in addition to amphetamine. More than 68% of the suicidal and homicidal was occurred in age group of 16 - 25 year and these percentage decreased with increased the age.

Conclusion/Discussion: A high rate of suicidality and homicides, especially among those under 35, was connected to an increase in fatalities involving amphetamines and other drugs (more than 4-fold between 2018 and 2020). Suicide deaths have been linked to high average blood amphetamine concentrations. Ethanol, THC, and cathinone were the most frequently used substances in combination with amphetamine. Additional research is needed to determine the relationship between tissue amphetamine concentrations and death, as well as the effect and treatment options for poisoning produced by combination of amphetamine with ethanol, TCH, and cathinone.

POSTER

P-076: Demographics and Vitreous Chemistry Results of Hypothermia Deaths Due to 2021 Winter Storm Uri in Harris County, Texas

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Background/Introduction: In February 2021, Winter Storm Uri caused widespread issues in the state of Texas including record-breaking power outages and dangerously low ambient temperatures. This put Harris County residents at high risk of hypothermia and death, especially those individuals who were homeless or had pre-existing medical conditions. Previous studies have indicated that high levels of glucose in vitreous humor as well as the presence of ethanol may be associated with death by hypothermia; however, these findings have not been replicated in other studies.

Objectives: This research investigated hypothermia deaths in Harris County, Texas to determine the demographic information, compare vitreous chemistry results to normal ranges, determine the average blood ethanol results, and determine if there are any differences between hypothermia deaths during the 2021 winter storm versus other hypothermia deaths in the previous five years.

Methods: Data were obtained for 43 hypothermia deaths in Harris County from December 2016 to February 2021 including vitreous chemistry results, blood ethanol results and demographic information such as age, race, sex, homelessness, and medical history. Sodium, chloride, potassium, urea nitrogen, creatinine and glucose results were compared to normal ranges to identify any trends and determine the prevalence of abnormal results. Data from deaths prior to February 2021 were compared to deaths during Winter Storm Uri to determine any notable differences.

Results: During Winter Storm Uri, 22 people died of hypothermia in Harris County, comprising 51% of all hypothermia deaths since December 2016. Other than gender, the demographic profile of individuals who died in Winter Storm Uri was different than previous hypothermia deaths. Homelessness was common amongst hypothermia victims prior to this event (76%), but only 27% of storm-related decedents were homeless. These findings are not surprising given the frigid indoor temperatures experienced during widespread power outages. Decedents who died prior to the winter storm were 38% White, 33% Black and 29% Hispanic, but during the storm, racial demographics shifted, as 55% were Black, followed by 36% White and 9% Hispanic. The elderly were disproportionately impacted as 50% of decedents during the storm were over 70 years old, compared to 14% in prior years. Cardiovascular disease was three-times more likely in storm-related decedents (68%) than in other hypothermia deaths (24%). Among all hypothermia deaths from 2016-2021, the average vitreous chemistry results were within normal ranges for sodium (143 mEq/L), potassium (9.4 mEq/L), chloride (115 mEq/L), and glucose (73 mg/dL), but not urea nitrogen (22 mg/dL) and creatinine (0.2 mg/dL). Approximately one-third of cases had elevated glucose (>80 mg/dL), but half of those were from known diabetics. Abnormal urea nitrogen results (68%) were more often observed in storm-related deaths than other hypothermia deaths (25%). Abnormally low creatinine results were also observed across both groups. Outliers for all analytes could often be attributed to documented pre-existing conditions such as diabetes or renal failure. There was no difference in alcohol positivity rates, as 39% were positive during the storm compared to 40% prior. Similarly, there was no significant difference in blood ethanol concentrations; the average ±2SD concentration in deaths during the storm was 0.198 ± 0.184 g/100 mL and 0.169 ± 0.171 g/100 mL prior.

Conclusion/Discussion: There was a significant increase of hypothermia deaths in Harris County during the 2021 winter storm compared to the previous five years, where the majority of these individuals were not homeless but were more advanced in age and had high rates of cardiovascular disease. Most vitreous chemistries were within normal ranges, but there was an increase in the prevalence of high urea nitrogen results during the winter storm.

POSTER

P-077: Urine drug screening in the era of designer benzodiazepines: results of three immunoassay platforms and comparison to LC-QTOF-MS

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Background/Introduction: We investigated the presence of designer benzodiazepines in 35 urine specimens obtained from emergency department patients undergoing urine drug screening on clinical indication. All specimens screened positive for benzodiazepines while confirmatory testing using a 19-component liquid chromatography-tandem mass spectrometry (LC-MS/MS) panel showed no benzodiazepines at detectable levels.

Objectives: The objective of this study was to investigate potential false positive screening results obtained after confirmatory testing was performed. Suspected designer benzodiazepines not included in confirmatory testing panel were screened via targeted high-resolution MS-TOF. Cross reactivity to other on market immunoassays were also tested against suspected designer benzodiazepines in study.

Methods: All specimens were obtained from emergency departments of a single US Health system (Michigan). Following clinically ordered drug screening using Abbott ARCHITECT *c* assays and lab-developed LC-MS/MS confirmatory testing, urine specimens were screened for amphetamines, barbiturates, benzodiazepines, cocaine, methadone, opiates, and THC using assays from two additional manufacturers (Roche cobas c502, Siemens Dimension Vista). Specimens were then further screened by liquid chromatography quadrupole time-of-flight mass spectrometry (LC-QTOF-MS); high-resolution mass accuracy m/z for precursors and fragments and retention time data were queried to identify presumptively positive species. The scope of analysis included a wide array of both prescription and designer benzodiazepine compounds, but not including metabolites of the designer benzodiazepines.

Results: Following benzodiazepine detection using the Abbott ARCHITECT (cutoff 300 ng/mL using oxazepam calibrators), benzodiazepines were subsequently detected in 28/35 and 35/35 urine specimens, respectively, using Siemens (cutoff 200 ng/mL, lormetazepam calibrators) and Roche (cutoff 100 ng/mL, nordiazepam calibrators) assays. Semi-quantitative analysis of the 7 specimens with concentrations below the Siemens assay cutoff revealed approximate cumulative benzodiazepine concentrations of 143-196 ng/mL. Targeted LC-QTOF-MS showed the presumptive presence of at least one non-FDA approved benzodiazepine (including those used outside the US and true designer benzodiazepines with no approved medical use) in 30/35 specimens: flubromazolam (12/35), flualprazolam (11/35), flubromazepam (2/35), clonazolam (4/35), etizolam (9/35), metizolam (5/35), nitrazepam (1/35), pyrazolam (1/35). Two or three designer benzodiazepines were detected concurrently in 12/35 samples. Medazepam, rarely prescribed in the United States, was presumptively identified in another specimen, resulting in 31/35 specimens with at least one benzodiazepine identified by LC-QTOF-MS. Among the four patients who showed no benzodiazepines by targeted LC-MS/MS or LC-QTOF-MS, one stated use of Xanax (alprazolam); clinical presentations for the others were not conclusive. Designer benzodiazepines were detected exclusively in the absence of any prescribed benzodiazepines, suggesting screening assays were cross-reactive with the former. Among the designer benzodiazepines detected, including triazolobenzodiazepines and thienodiazepines, multiple have uncharacterized cross-reactivity with the screening assays used: flualprazolam (Roche); flubromazolam, flualprazolam, flubromazepam, clonazolam, etizolam, metizolam, and pyrazolam (Siemens and Abbott).

Conclusion/Discussion: Urine benzodiazepine screening assays from three manufacturers were cross-reactive with multiple non-US FDA-approved benzodiazepines, suggesting the utility of screening methods for detecting designer benzodiazepine use. Clinical and forensic toxicology laboratories using traditionally designed LC-MS/MS panels may fail to confirm the presence of non-US FDA-approved benzodiazepines detected by screening assays, risking inappropriate interpretation of screening results as false-positives. As prescribed and unprescribed benzodiazepine use grows, laboratories should consider expanding their confirmatory test panels to include non-US FDA-approved benzodiazepines and/or performing untargeted screening with structural identification (e.g., LC-QTOF-MS) to ensure appropriate interpretation of drug screening results.

P-078: Detection of Eutylone in Chronic Pain and Behavioral Health Populations

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Background/Introduction: Eutylone is classified as a synthetic cathinone, a group of Novel Psychoactive Substances (NPS) that act as central nervous system (CNS) stimulants. First identified on the DEA Emerging Threat Report in 2019, eutylone has quickly become the most detected cathinone derivative. Clinicians in chronic pain and behavioral health settings should be aware of eutylone as a potential drug of abuse, especially when ingested with alcohol or other CNS stimulants.

Objectives: The goal of this study was to evaluate the prevalence of eutylone in a chronic pain and/or behavioral health clinical setting and to better characterize its potential impact on patient care.

Methods: This study was IRB approved. The scope of testing for all samples received in the laboratory depends upon physician request; test offerings include routinely monitored healthcare compliance medications and illicit drugs, including NPS. Eutylone was analyzed in the laboratory as part of a larger NPS panel, which includes designer benzodiazepines, designer opioids, synthetic cannabinoids, synthetic stimulants, and other NPS analytes. Analytes were extracted from hydrolyzed urine using a liquid-liquid extraction followed by evaporation and reconstitution in mobile phase. Samples were injected onto a liquid chromatography/tandem mass spectrometry (LC-MS/MS) instrument consisting of a Shimadzu Prominence HPLC and Sciex API 4000 MS/MS. The mass spectrometer was operated in positive electrospray ionization mode for scheduled multireaction monitoring (sMRM) analysis. Chromatographic separation was achieved using a Restek Biphenyl column. Results were reported qualitatively with a detection limit of 2 ng/mL. Eutylone results were evaluated from July 2020 through early April 2021 from samples received across 43 states. During this 9 month period, providers requested 123,304 tests for synthetic stimulants, of which eutylone is a component. Positive eutylone results were summarized for routine demographic information as well as additional relevant positive results. Co-positive analytes discussed in this study were analyzed by separate validated methods.

Results: Seventy-two (72) positive eutylone results were reported over the time period evaluated. Of these patients, 34 were female and 38 were male. The median age was 37, with patients ranging from 19-68 years old. Positive results were reported from patients residing in 14 different states, with the highest concentrations in Louisiana (32%) and Illinois (14%).

The chart below details co-positive analytes observed in the 72 eutylone positive patient samples:

Analyte	Sample number (%)
d-Methamphetamine	34 (47%)
Amphetamine	32 (44%)
MDMA/MDA	6 (8%)
Cocaine/Benzoylecgonine	23 (31%)
Heroin/6-Acetylmorphine/6-Acetylcodeine	7 (9%)
Fentanyl/Norfentanyl	18 (25%)
Buprenorphine/Norbuprenorphine	34 (47%)
Ethyl glucuronide/Ethyl sulfate	20* (31%)
*Only 63 of the 72 positive eutylone samples were tested for EtG/EtS; percentage reflects those samples only	

Conclusion/Discussion: Eutylone is an analyte of concern for clinicians in chronic pain and behavioral health settings. In this study, eutylone was detected in patients from a variety of age groups across 14 states with no gender bias, which suggests its abuse is not limited to a specific population. Eutylone is frequently detected in the presence of other prescription and illicit drugs, as well as alcohol. Almost half of all positive eutylone samples were also positive for methamphetamine, while almost one-third were positive for cocaine. The ingestion of multiple stimulants increases the risk of an adverse cardiovascular event. Of the 63 positive eutylone samples also tested for alcohol markers, one-third were positive. Co-ingestion of eutylone with alcohol may mask the effects of alcohol intoxication, which may result in excessive drinking and/or alcohol poisoning. The availability of comprehensive analytical testing for NPS compounds, including eutylone, provides a valuable tool for clinicians to utilize to support patient treatment plans.

P-079: A non-fatal intoxication case with MDMA and alcohol consumption

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Background/Introduction: MDMA or 3,4-méthylènedioxyméthamphétamine is well known as an illegal recreational drug widely used for its empathetic and entactogenic effects. It is frequently associated with alcohol and other drugs at festive events. For ten years, a resurgence in its use has been described with the epidemiological data of French addiction centers, with an increase in the number of cases of intoxications and deaths.

Objectives: We report a non-fatal intoxication case with a 21-year-old man admitted in intensive care unit after consumption of MDMA and alcohol in a binge drinking situation (about fifteen pints of beer and some whiskey), at a private party.

Methods: Collected blood, urine and hair samples (1 cm) and the product ingested were submitted to the laboratory for toxicological investigations. Screenings using immunochemical, enzymatic, LC-DAD/MS, LC-MS/HR and GC-MS were performed.

Results: At the arrival of the emergency medical service, the patient was comatose (Glasgow 3), and presented with a generalized convulsive crisis which was treated by clonazepam. Upon admission to the intensive care unit, the patient was hyperthermic at 39°C, hypotensive and had pupils in reactive bilateral mydriasis. Biological results highlighted a metabolic acidosis (lactatemia 14 mmol/L), low increase in ASAT (ASpartate AminoTransferase) and blood creatine and increase in troponin (10N) without cardiac abnormalities on electrocardiogram. The patient had several episodes of hypoglycemia, degradation of liver function with a TP at 35% (Factor V at 20%), cytolysis that reached its zenith 48 hours after admission (with values at more than 40 times normal (ASAT = 1496 IU/L and ALAT (Alanine AminoTransferase) = 1543 IU/L)), and no sign of cholestasis. After four days of hospitalization, liver cytolysis with ASAT and ALAT persisted at 35N and the patient was transferred to the gastroenterology department. Toxicological screenings revealed the presence of MDMA and plasma alcohol of 2.19 g/L. Measured MDMA concentrations in plasma, urine and hair were elevated: 68644 ng/mL, 73250 ng/mL and 112000 pg/mg, respectively. Its metabolite MDA concentrations were 547 ng/mL in plasma, 690 ng/mL in urine, and 1410 pg/mg in hair. Chemical testing of the ingested drug indicated that only MDMA was present with a purity of 53%.

Conclusion/Discussion: High variability in plasma concentrations of MDMA in non-fatal intoxications is described in the literature. To our knowledge, this is the first case reported with high concentrations of MDMA in plasma, urine and hair. The clinical symptoms were related to its serotonin and dopaminergic activities, with hyperthermia and liver cytolysis related to acute liver failure which were easily reversible. The length of the hair did not allow for segmentation to assess the patient's MDMA consumption profile or help the interpretation of an external contamination by sweat (which was highlighted by the analysis of decontamination baths). However, it showed endogenous impregnation of MDMA and MDA at concentrations much higher than post-mortem hair concentrations and similar to the highest concentrations described in intensive consumers. There is an increase in cases of amphetamine poisoning in France and in particular MDMA, confirming the need to maintain information systems, prevent and reduce risks for consumers and medical treatment in hospitals.

POSTER

P-080: Sensitive Quantitation of Per-and Poly-Fluoroalkyl Substances (PFASs) in Serum

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Background/Introduction: Per- and polyfluoroalkyl substances (PFAS) are pervasive compounds used in a variety of industrial applications and found in a wide range of everyday consumer products (e.g., cookware, stain repellant, flame-retardant, coatings, and even drinking water). PFAS are considered environmental factors due to their persistent and bioaccumulating nature. Given their prevalence and ubiquity, there is a critical need to develop quantitative tools capable of accurately and precisely detecting low levels of PFAS in biological fluids, to inform the extent of their bioaccumulation and overall impact on the human body.

Objectives: To develop a quantitative workflow for the analysis of 22 PFAS in serum was developed using the SCIEX QTRAP 6500+ System. The method provides a fast analytical method capable of accurately quantifying sub-nanogram per mL levels of PFAS in the human body.

Methods: A total of 22 PFAS and 15 mass-labeled internal standards were purchased from Wellington Laboratories (Guelph, ON, Canada) and prepared using Baker's HPLC-grade methanol. A series of 9 calibrator solutions ranging from 0.01-100 ng/mL were prepared using blank, charcoal-stripped, fetal bovine serum. This range of concentrations encompasses the levels of PFAS typically observed in humans after exposure concentrations. PFAS were extracted from 50 μL of fortified serum by using a protein precipitation procedure. Calibration curves were generated for each of the PFAS in the panel and plotted across 9 calibration levels ranging to evaluate the linearity of the method. Analytes were chromatographically separated using a Phenomenex Gemini® C18 column (50 x 2 mm, 3 μm, 00B-4439-B0) at 25°C. Mobile phases used consisted of ammonium acetate in water (mobile phase A) and formic acid in methanol (mobile phase B) prepared from HPLC and LC-MS/MS grade solvents. A delay column was placed between the autosampler and LC pumps to isolate PFAS contamination leaching from the LC system components and minimize the risk of system-related PFAS interfering with real signals from the sample during the analytical run. The LC flow rate was 0.6 mL/min and the total run time was 6.5 min. The injection volume was 10 μL. A single acquisition method consisting of 57 MRM transitions (42 for the PFAS and 15 for the mass-labeled internal standards) was created.

Results: The most critical measure was the inclusion of a delay column to trap ambient and system-related PFAS and ensure they were retained away from the sample signals. The addition of the delay column and the modifications made to the LC system components together minimized the impact of system-related PFAS contamination and ensured the analytical integrity of this quantitative workflow. The results showed a high level of consistency and precision, as evidenced by the acceptance criteria (20% or less) of all the ion ratios across the calibration series ranging from 0.5 (LLOQ) to 100 ng/mL. The levels quantified in this study are well within the range of PFAS concentrations detected in human following exposure. In addition, the assay showed excellent reproducibility, precision, accuracy and linearity, with an LLOQ of 0.5 ng/mL, LOD of 0.1 ng/mL and an R^2 of greater than 0.99 for the vast majority of the PFAS in the panel with the exception of PFBS and PFODA. Precision and accuracy observed at the LLOQ were <20% and 80.98-116.49%, respectively.

Conclusion/Discussion: A robust and sensitive workflow for the detection of PFAS in serum samples using the SCIEX QTRAP 6500+ System was successfully developed. Overall, the developed method provides a robust and accurate method for bio-monitoring of low-levels of PFAS in biological fluids. Therefore, the presented workflow is readily adaptable for high-throughput toxicology investigations aimed at determining the extent of PFAS bio-accumulation and its broader impact on human health.

POSTER

P-081: Postmortem Toxicology in Kentucky: Looking Back at 2020

Kevin G. Shanks, M.S.[1]*, George S. Behonick[1], and William Ralston, M.D.[2]

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Introduction: Drug overdoses and drug-related fatalities in the United States have increased over the past several years. According to the Centers for Disease Control and Prevention (CDC), in 2019, the Commonwealth of Kentucky ranked seventh in the country for drug overdose death rates (32.5 deaths per 100,000 people). According to preliminary data released by the CDC in 2021, there was a 29% increase in US drug-related deaths from October 2019 to September 2020. In Kentucky, overdose deaths increased by 50% during that time frame.

Objectives: Since all postmortem toxicology for Kentucky is completed by our forensic toxicology laboratory, the objective was to determine the prevalence of common substances detected in postmortem blood during routine death investigation in Kentucky during 2020. We did not study the certified cause of death determination made by the forensic pathologist. A version of this data was presented at the Kentucky Coroner's Association Conference in April 2021.

Methods: Samples for toxicological analyses were obtained at autopsy by a forensic pathologist and collected in tubes containing sodium fluoride as a preservative. Targeted screening was completed by a combination of liquid chromatography quadrupole time of flight mass spectrometry (LC-QToF-MS) for drugs and drug metabolites and headspace gas chromatography with flame ionization detection (HS-GC-FID) for volatiles. Confirmatory analyses, such as fentanyl, cocaine, methamphetamine, and designer opioids, were completed by liquid chromatography with triple quadrupole mass spectrometry (LC-MS/MS) or gas chromatography with mass spectrometry (GC-MS). Cases which did not include blood as a specimen were not included in the data set.

Results: 6,188 cases (comprised of 18,798 specimens) from Kentucky were analyzed by the toxicology laboratory in 2020. Of these 6,188 cases, 5,907 contained a postmortem blood specimen. The top 5 most encountered analytes in postmortem blood in Kentucky in 2020 were fentanyl (n=1,633; mean, 20.4 ng/mL; range, 0.1-801 ng/mL), methamphetamine (n=1,017; mean, 1,186 ng/mL; range, 50-96,463 ng/mL), ethanol (n=965; mean, 0.139%; range, 0.020-0.646%), 4-anilino-N-phenethylpiperidine (4-ANPP) (n=958, reported qualitatively), and 11-nor-9-carboxy-delta-9-tetrahydrocannabinol (THC-COOH) (n=927; mean, 42.0 ng/mL; range, 3.0-505 ng/mL). Other commonly detected substances were gabapentin (n=759), THC (n=718), amphetamine (n=607), oxycodone (n=328), and morphine (n=310). Of particular note, there were two noticeable spikes in positive casework for fentanyl and methamphetamine occurring in May and December 2020.

Conclusions: Fentanyl and methamphetamine were the two most common substances detected in postmortem blood toxicology in the Commonwealth of Kentucky during 2020. Gabapentin is commonly found in postmortem blood toxicology casework. As drug-related deaths and overdoses are increasing across the US, it is prudent that a forensic toxicology laboratory assess drug trends and prevalence for the locations which submit casework to them.

POSTER

P-082: The Ever-Changing Scope of Synthetic Cannabinoids in Toxicology Casework II (2016-2020)

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Introduction: Synthetic cannabinoid receptor agonists are laboratory synthesized compounds that bind to cannabinoid receptors CB_1 and CB_2. Over the last decade, synthetic cannabinoids have been implicated in intoxication, hospitalizations, and deaths across the United States. Since 2011, the Federal government has passed legislation to control 43 synthetic cannabinoids as Schedule I controlled substances. AIT Laboratories/Axis Forensic Toxicology began testing for synthetic cannabinoids in 2010. During the last decade, the number of compounds on the drug market has drastically changed, which has led to a varying scope of testing in the modern forensic toxicology laboratory.

Objectives: At the 2016 SOFT conference in Dallas, Texas, we presented data describing the changing scope of synthetic cannabinoids in toxicology casework for the previous 5 year time period (2011-2015). The objective of this presentation is to describe the changing breadth of synthetic cannabinoids in toxicology casework over the last 5 years (2016-2020). A year by year depiction of the overall trends in scope of synthetic cannabinoids detected in blood specimens will be presented.

Methods: All blood specimens were prepared via a liquid-liquid extraction into ethyl acetate or iso-amyl alcohol in hexane or a protein precipitation extraction with acetonitrile. Instrumental analyses were completed by liquid chromatography with triple quadrupole mass spectrometry (LC-MS/MS). Method validation was completed according to in-house method validation standard operating procedures. Attributes studied during the validation process included linearity, accuracy and imprecision, carryover, exogenous drug interferences, and matrix selectivity/ion suppression. A post-hoc review of toxicology results in the laboratory information management system (LIMS) was completed in 2021 and data was compiled.

Results: During the 5 year time period evaluated, the overall positivity rate for synthetic cannabinoids in analyzed blood casework was 16.3%, but varied from year to year (9.0-27.6%). A total of 24 different synthetic cannabinoids and metabolites were detected during this time period across 21 states. In 2016, 2017, and 2018, 5F-ADB was the most prevalent compound detected in the synthetic cannabinoid testing (38.8%, 52.7%, and 80.4%, respectively), while in 2019, 4F-MDMB-BINACA was the most prevalent compound (42.3%). During 2020, the most prevalent compound reported in postmortem toxicology casework for synthetic cannabinoids testing was 5F-MDMB-PICA (60.0%). During this time period, the Federal government passed four pieces of legislation which were used to control various synthetic cannabinoids as Schedule I controlled substances. From 2016 to 2017, the absolute number of different compounds detected decreased, but we observed no correlation between pending/passed legislation controlling compounds and synthetic cannabinoids appearing or disappearing in casework.

Conclusions: After increases were observed in the absolute number of synthetic cannabinoid compounds detected 2011-2015, the number of synthetic cannabinoids detected in toxicology casework decreased over the last five years (2016-2020). The main substances detected were 5F-ADB, 4F-MDMB-BINACA, and 5F-MDMB-PICA. In order to sufficiently aid in investigations for postmortem and human performance toxicology matters, the modern forensic toxicology laboratory must be aware of the ever-changing scope of synthetic cannabinoids on the market. This current data set can be added to our previous presentation on synthetic cannabinoid prevalence to form an entire decade of synthetic cannabinoid trends data (2011 – 2020).

POSTER

P-083: Ritalinic Acid in Urine; Effect of Patient Age

Michaela Roslowski[1], Sheng Feng[2], Jeffery Enders[3], Erin Strickland[4], **Gregory McIntire[1,4]***

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Introduction: Methylphenidate has been used to treat symptoms of Attention Deficit Hyperactivity Disorder (ADHD) for over 50 years. Various reports suggest that diagnosis of ADHD and subsequent treatment with stimulant drugs such as methylphenidate have increased to as much as 15% of the population. Inasmuch as methylphenidate is a stimulant, it has been and continues to be abused. Urine drug testing (UDT) is often employed to help assess patient adherence to chronic drug prescriptions. Since 80% of the oral dose of methylphenidate is excreted as ritalinic acid (RA) in urine the resulting concentrations can be relatively high making identification of diversion and/or abuse difficult. The work reported herein was directed at defining "normal" urine levels of ritalinic acid for patients prescribed methylphenidate.

Objectives:

- To estimate "normal" urine concentrations of RA from patients prescribed Methylphenidate and testing positive for RA in UDT.
- To determine any differences in RA concentrations from different age groups and sex.

Methods: Ritalinic acid is part of a larger test panel. Details of the full method and validation can be found in an earlier report by Enders et al. (*Journal of Applied Laboratory Medicine*, 2018, 2(4), 543-554.) Over 16,000 test results were examined to estimate normal ranges of RA in urine.

Data Analysis
The test results for RA were curated as follows:

1. Only patients who were prescribed methylphenidate (e.g., Ritalin®, Concerta®, etc.) and tested positive for RA were included.
2. Patients testing positive for any illicit drugs were excluded.
3. Patients that did not test consistent with any other prescription(s) were excluded.
4. Patients who failed sample validity testing (e.g., pH, creatinine, and specific gravity) were excluded.
5. Patient samples without a UDT quantitative result (i.e., >ULOL) were not included.

This filtering process took the original 16,055 data points for ritalinic acid down to 10,738 data points post cleaning.

Results: A nonparametric Mann-Whitney U test indicates there is a significant difference between the mean of the RA concentration in patients between 6 and < 18 years (14,034 ng/mL) and that in patients from 18 to < 65 years and older (8,924 ng/mL) ($p < 0.001$). This does not seem to be a function of body weight, creatinine or daily dose. While creatinine concentration does appear to increase with age until about age 18, it is neither a big change nor unexpected. Specific Gravity and pH are consistent across all ages.

Discussion: Box and whiskers plots of the RA data curated as discussed in the methods section are an attempt to define "normal" ranges of RA from methylphenidate patients. While the overall range (all data) is interesting, the box and whiskers plots representing patients between 6 and < 18 years old demonstrates the difference between this unique population and both younger and older age groups. On average, these patients are dosed 10 mg/day, less than older patients even though their median concentration of RA is almost two fold greater than that of the older patients. Lastly, there are no differences between male and female patients with the possible exception of patients over 65 where the median concentrations are 8,535.5 ng/mL for males and 9,648.0 ng/mL for females. Both groups exhibited average doses of 20 mg/day. Part of the focus for this work is to aid physicians in determining patient adherence. To be successful, RA outliers should be readily identified from a comparison of the data. The ability to quickly compare UDT results, without further mathematical manipulation, from a large test population should help physicians determine patient adherence from their UDT data.

Disclosures: The authors do not have any financial interest in this work.

POSTER

P-084: Amphetamine by Age with Prescriptions

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Introduction: Amphetamine (Adderall, Benzedine, etc.) has been used to treat symptoms of Attention Deficit Hyperactivity Disorder (ADHD), narcolepsy, and obesity for over 80 years. Inasmuch as amphetamine is a stimulant, it has been and continues to be abused. Urine drug testing (UDT) is often employed to help assess patient adherence to chronic drug prescriptions. The work reported herein was directed at defining "normal" urine levels of amphetamine for patients prescribed amphetamine.

Objectives: To estimate "normal" urine concentrations of amphetamine from patients prescribed amphetamine and testing positive for amphetamine in UDT. To determine any differences in amphetamine concentrations from different age groups and sex.

Methods: Amphetamine is part of a larger test panel. Details of the full method and validation can be found in an earlier report by Enders et al. (*Journal of Applied Laboratory Medicine*, 2018, 2(4), 543-554.)

Data Analysis

The test results for amphetamine were curated as follows:

1. Only patients who were prescribed amphetamine and tested positive for amphetamine were included.
2. Patients testing positive for any illicit drugs were excluded.
3. Patients that did not test consistent with any other prescription(s) were excluded.
4. Patients who failed sample validity testing (e.g., pH, creatinine, and specific gravity) were excluded.
5. Patient samples without a UDT quantitative result (i.e., >ULOL) were not included.

Results: A nonparametric Mann-Whitney U test indicates there is a significant difference between the median amphetamine concentration in patients ≤15 years (4181.0 ng/mL) and that in patients ≥16 years (3091.0 ng/mL) ($p<0.001$). The difference between the age groups was not found to be a function of body weight, creatinine or daily dose. While creatinine concentration does increase with age until about age 18, the change is neither significantly large nor unexpected. Specific Gravity and pH are consistent across all ages.

Discussion: Table 1 shows the median for the entire data set as well as medians for patients ≤15 years and of patients ≥16 years. It is an attempt to define "normal" ranges of amphetamine from amphetamine patients. While the overall range (all data) is interesting, medians representing patients ≤15 years and patients ≥16 year demonstrate the difference between these unique populations. On average younger patients are dosed the same as older patients at a median dose of 60 mg/day. Despite this their median concentration of amphetamine is nearly 35% greater than that of the older patients. Additionally, no discernible difference was found in amphetamine concentrations between male and female patients. In collection of patient demographics on gender, or biological sex, some individuals identified themselves as "Other" (see Table 1). Of those that identified as such, eight out of nine were over 16 years old (and under 55) with concentrations that mirrored the overall ≥16 year old group data. All other parameters (i.e. pH, creatinine, etc.) were consistent across the entire population. Part of the focus for this work is to aid physicians in determining patient adherence. To be successful, amphetamine outliers should be readily identified from a comparison with Table 1. The ability to quickly compare UDT results without further mathematical manipulation to results from a large test population should help physicians determine patient adherence from their UDT data.

Disclosures: The authors do not have any financial interest in this work.

Table 1. Concentrations for all patients, patients ≤15 years, and patients ≥16 years.

Group	N	2.50%	25%	Median	75%	97.50%
All Samples	9468	198	1268	3166	7231	27087
Male Samples	4043	195	1245	3003	6878.5	25501
Female Samples	5416	201	1286	3296	7542	28966
Other	9			2697		
≤15 yr	782	222	1815	4181	8270	28826
≥16 yr	8685	196	1235	3091	7080	26829

POSTER

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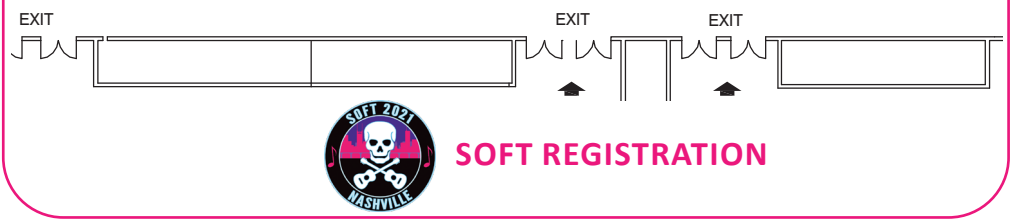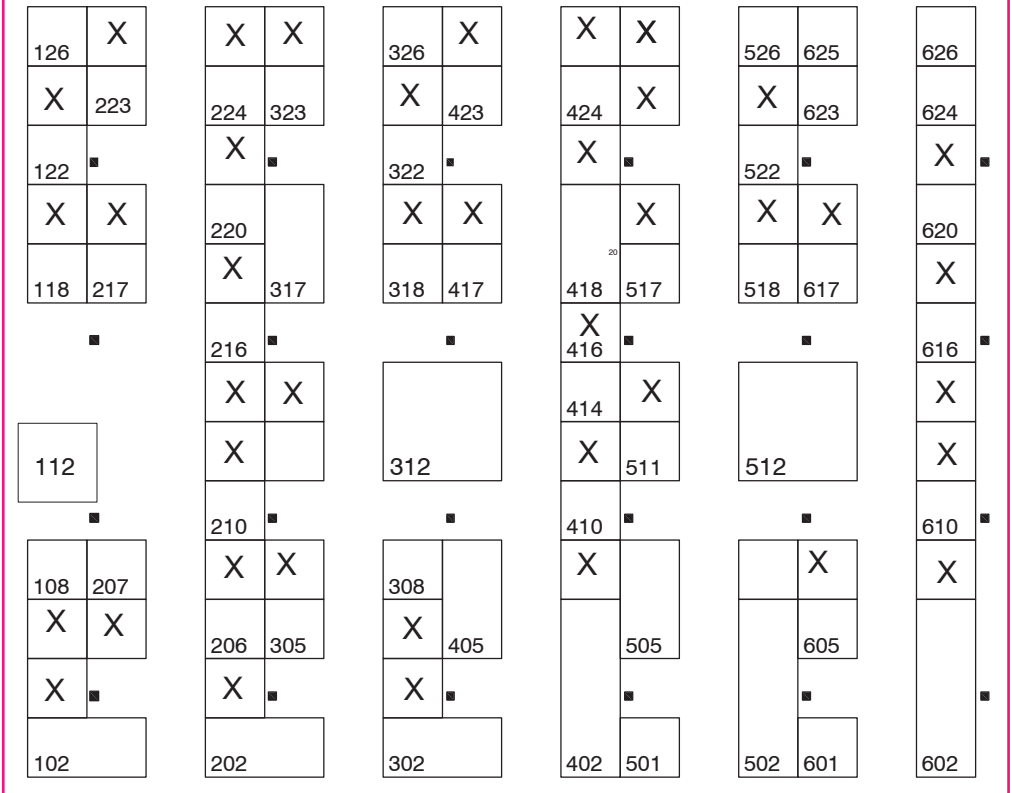
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Over 700,000,000 pipette tips have been washed and reused by Grenova, proving the safety and effectiveness of the only reuse technology available today. Grenova's tip washers were developed and have been tested in CLIA- and CAP-approved labs on multiple assays without carryover. In addition, they are implemented in the NIH, NCI and CDC. Grenova products routinely receive 5 out of 5 stars in customer reviews and have consistently demonstrated the following results:

- Washing and reusing pipette tips with Grenova's solution reduces plastic pipette tip consumption by 90% on average, resulting in significant cost savings while dramatically cutting plastic waste impacting the environment.

- Labs implementing a sustainable plan to safely wash and reuse pipette tips are controlling their most important inventory and avoiding pipette tip shortages.

- Labs leveraging Grenova's green technology create supply chain resiliency by solving the current distribution bottleneck that have resulted in pipette tip backorders due to the COVID-19 pandemic.

- Researchers have concluded that both the TipNovus and TipNovusMini each decrease a lab's carbon footprint by 93-95% every 10-minute wash cycle.

- Grenova's tip washers have saved the industry over $58,000,000 and reduced over 2,000,000 lbs. of plastic waste.

Contact Grenova to implement a sustainability plan in your lab to safely wash and reuse pipette tips as a way to control your most important inventory, reduce costs, and eliminate waste impacting the environment. Learn more about Grenova at www.grenovasolutions.com.

i3 Verticals Healthcare

i3 Verticals Healthcare, powered by Healthpac is a nationally recognized medical billing software company. A leader in medical coding, billing, and scheduling software development, we specialize in laboratory billing and revenue cycle management. Healthpac offers cloud and server-based solutions used by our nearly 30,000 providers nationwide. Notably, the state labs of Texas, Florida, South Dakota, and Nevada currently utilize our software solutions. www.healthpac.net.

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For more information on the i3 Healthcare legacy or to schedule a demonstration, contact:

Melinda Lewis

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We are an exclusively virtual community of clinical and forensic toxicologists, analytical laboratory professionals and toxicology students, driven by a single goal: to make clinical and forensic toxicology accessible to all.

We are from all over the world, and we serve our local communities as best as we can, but we don't always have the time or budget to attend professional meetings in remote parts of the world to present our research, our case studies, our analytical methods, or to learn the latest developments in our field.

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IMCS

Integrated Micro-Chromatography Systems, Inc. (IMCS) is a privately-held biotech company that designs, manufactures, and distributes biological reagents from next-generation recombinant proteins to micro-chromatography consumables. The company's two product lines, IMCSzyme® and IMCStips® , serve various clients, including clinical and forensic toxicology labs, academic research facilities, US federal government agencies, and life science companies in North America, Europe, and the Asia Pacific.

IMCSzyme®, the first genetically modified, industry-disrupting β-glucuronidase enzyme is designed to maximize the testing capabilities of laboratories by reducing hydrolysis times and increasing sample processing efficiency. The new IMCSzyme® RT is a second-generation β-glucuronidase formulated to hydrolyze drugs of abuse at room temperature in 15 minutes or less. It is the only purified β-glucuronidase enzyme in the market resistant to naturally occurring inhibitors in human urine samples. Learn how to reduce your sample costs while improving quality by contacting us here or emailing inquiries@imcstips.com.

As a leader in recombinant protein engineering, IMCS was recently awarded NIH SBIR funds to manufacture various glycosyltransferases. This grant will leverage the company's advanced manufacturing and research capabilities to expand the synthesis of glycosphingolipids and sialoglycans. This expansion provides IMCS with new product lines and critically needed biological reagents that were not previously available to the scientific community.

IMCStips® are advanced micro-chromatography products centered around patented dispersive solid-phase extraction technology for faster biochemical purification processes. IMCStips are utilized by academic laboratories and life science companies for purifying new proteins, antibodies, and enzymes, as part of drug target discovery and validation, drug development, drug screening and drug manufacturing.

INTEGRA Biosciences

Company Contact: Darren Hoover

Email: Darren.Hoover@Integra-Biosciences.com

Website Address: https://www.integra-biosciences.com/

Moving liquids in a precise and productive way is the core to many tasks of laboratory professionals in the life sciences industry. Since 1965, INTEGRA has been dedicated to developing solutions for pipetting and media preparation, which fulfills the needs of our customers in research, diagnostics and quality control. It is our passion to work side by side with our customers to understand their problems and answer their needs with innovative products. In order to do this, we maintain our own sales and support organizations in the USA, Canada, China, Japan, UK, France, Germany, Austria and Switzerland as well as a network of over 100 highly trained distribution partners worldwide.

INTEGRA's engineering and production teams in Zizers, Switzerland and Hudson, NH USA strive to develop and manufacture instruments and consumables of outstanding quality. In recent years, we have focused on completing our technologically advanced liquid handling product range, culminating in the launch of the ASSIST PLUS pipetting robot; a revolutionary concept that combines state of the art handheld pipettes with cutting edge robotics, freeing scientists from routine pipetting. We are proud to offer the widest product line of pipettes in the market, spanning a range from single channel mechanical pipettes up to 384 channel electronic bench-top platforms.

JEOL

JEOL led the development of ambient ionization mass spectrometry for forensic analysis with the introduction of the revolutionary AccuTOF™-DART® mass spectrometer in 2005. JEOL solutions for toxicology and drug chemistry include the current fourth-generation model, the AccuTOF™-DART® 4G mass spectrometer, the TQ4000 GC/triple quadrupole mass spectrometer system, the Q1500 single quadrupole GC/MS system, and the new AccuTOF GC-Alpha GC-high-resolution time-of-flight mass spectrometer system.

The combination of rapid sample preparation methods such as solid-phase microextraction (SPME) with the AccuTOF™-DART® 4G permits detection of trace levels of drugs in body fluids such as urine and saliva. PaperSpray® and Coated Blade Spray® options provide alternative ambient ionization solutions. The TQ4000 offers high sensitivity, ease of use, and the fastest-available SRM switching rate for screening and quantitation of a large number of target compounds. The AccuTOF GC-Alpha with new msFineAnalysis software is ideal for non-targeted compound identification.

JEOL is a leading global supplier of mass spectrometers, NMR, and electron microscopes used for scientific research and industrial applications. Learn more at www.jeolusa.com/forensics.

JusticeTrax

Please visit Jason Pressly and David Epstein in Booth 217

JusticeTrax is the premier forensic science management software company delivering all the tools a forensic operation needs to electronically manage processes, from receiving evidence, barcoding it, processing the evidence, recording analytical results and conclusions, and producing the final report, all while maintaining the tightest possible chain of custody.

Since our inception in 1995 we have worked diligently to understand both business and science aspects of operating and managing a forensic laboratory. Relying on a staff that includes ten forensic scientists with a combined experience of over 125 years in the field has resulted in our suite of carefully developed software products aimed at helping laboratories just like yours with the daily challenges faced regarding the receipt, processing, analyzing, and reporting on physical evidence.

Once a laboratory chooses JusticeTrax to be its partner for providing laboratory management they find these tenets in place: superior software design, features/benefits, functionality, and customizability; software flexibility, and ironclad reliable and responsive technical support whenever needed, and for which existing customers give their highest praise.

JusticeTrax' suite of software includes LIMS-plus, LIMS-plus DNA, Consumables Inventory Management System (CIMS), LIMS-plus Portal, and Indexer, providing casework and sample management, are implemented, configured and controlled by the customer agency's system administrator.

MilliporeSigma

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MilliporeSigma is the North America life science business of Merck KGaA, Darmstadt, Germany. Our portfolio spans more than 300,000 products enabling scientists in clinical and forensic laboratories to develop innovative solutions for their scientific challenges whether it is to get ahead of the opioid epidemic or to optimize your wellness testing. From Cerilliant certified reference materials, Supelco analytical products, to Milli-Q water systems our portfolio and expertise come together to collaborate with your lab and help you ensure your results are accurate, every time.

NEOGEN

NEOGEN® has supplied the Forensic Toxicology industry for more than 30 years and has become a trusted partner for drug screening solutions. We offer a wide range of ELISA tests to provide solutions for drugs of abuse, including designer drugs and emerging drugs. Our solutions ensure confidence in your testing and provide reliable results.

Our comprehensive line of drug detection assays includes more than 115 ELISA kits that can be used to screen more than 300 drugs and their metabolites in various forensic matrices, including oral fluid, whole blood, urine, serum, plasma, meconium, and others. Our testing solutions are designed for high throughput demands, so you can count on a cost-effective, optimized workflow without sacrificing accuracy or time.

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OraSure Technologies manufactures industry specific oral fluid devices in conjunction with other innovative technologies designed to detect or diagnose critical medical conditions. Our diverse product lines include rapid tests for HIV, HCV and COVID-19 antibodies, COVID and influenza antigens, multiple testing solutions for detecting drugs of abuse in various sample matrixes, and numerous oral fluid sample collection methods that include stabilization and sample preparation products for molecular diagnostic applications.

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Randox

Randox has over 35 years' experience in the diagnostic market with a heavy focus on R&D, and is dedicated to advancing forensic, clinical and workplace toxicology. This focus has led to the development of technology, which is at the forefront of advanced global diagnostics, and trusted by market leaders to deliver accurate and reliable results.

Randox Toxicology is a market leader in the development of new assays and technology in the field of toxicology, aiming to minimise laboratory workflow constraints whilst maximising the scope of quality drug detection. We are the primary manufacturer of Biochip Array Technology and ELISAs for forensic, clinical and workplace toxicology.

Biochip Array Technology is an immunoassay testing platform allowing for the simultaneous multi-analyte testing of a panel of related tests with a single sample. The technology works by combining a panel of up to 44 related tests on a single Biochip with a single set of reagents, controls and calibrators. Competitive chemiluminescent immunoassays are employed, and a light signal is generated from each of the test regions on the Biochip. This is detected using digital imaging technology and compared to that from a calibration curve.

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Booth # 108

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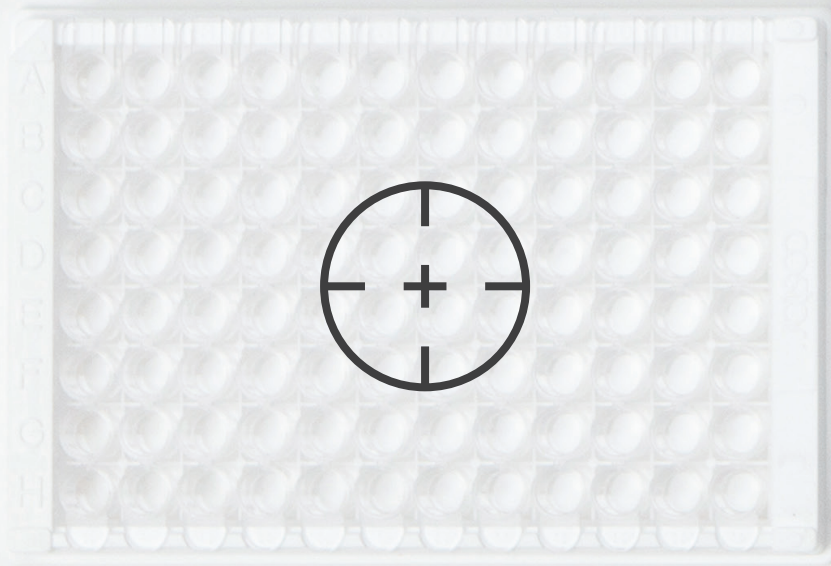
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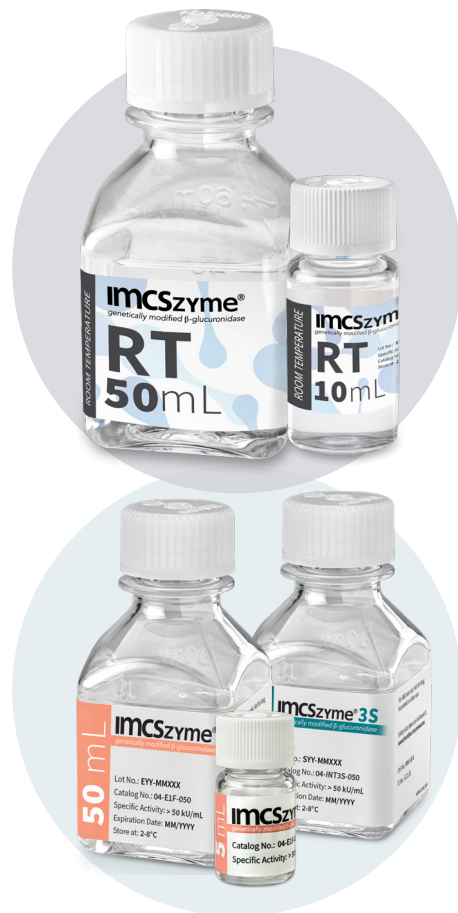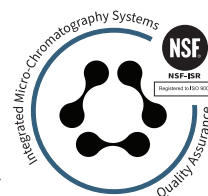
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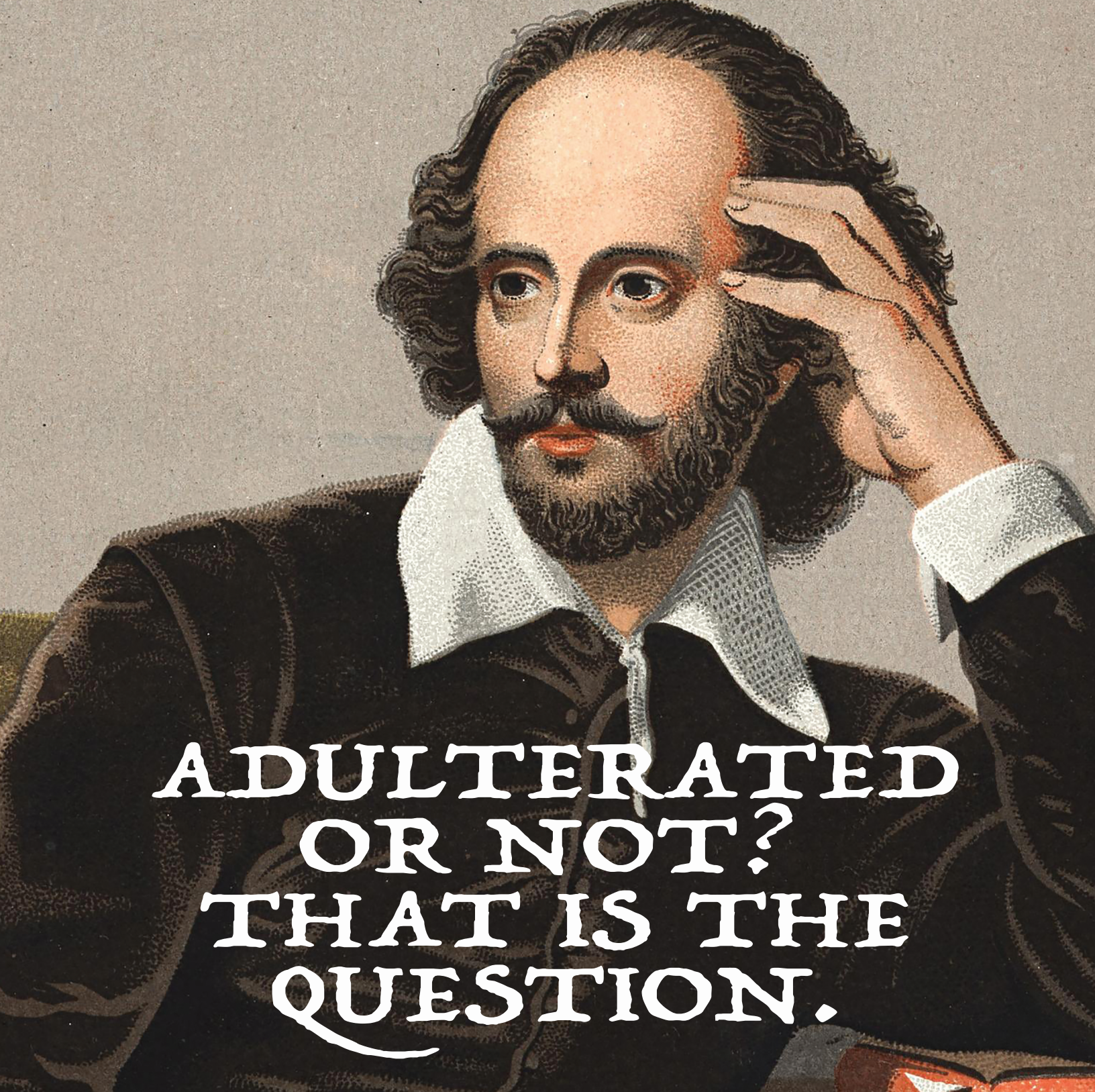
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