PROGRAM & ABSTRACTS

WELCOME TO SOFT 2024!

Welcome to the Gateway City! We are so pleased, and so excited, to have you joining us for this incredible meeting. If you are a first-time attendee, welcome to the SOFT family! If you are a veteran of many meetings, welcome back! Either way, we hope that this meeting exceeds your expectations for scientific content, networking, and fun!

Show Me the Science! Our program aims to do just that. The half-day workshops include topics on psychedelics, civil cases, doping, impairment, ASB standards, breath alcohol, the post-fentanyl era, lab development, cannabinoids, oral fluid, AFB certification, sample prep, LIMS, and building a diverse organization. On Wednesday morning, our plenary speaker, Ben Westhoff, investigative journalist, and author of the best-selling Fentanyl Inc., kicks off our scientific sessions. These sessions continue through Friday afternoon. Wednesday and Thursday, browse the poster sessions held off of the exhibit hall and visit with our exhibitors.

For relaxation and rejuvenation during the meeting, you can join in a yoga session from 6-7 am in the Conductor Room. These complimentary sessions are new this year and open to all regardless of experience. Or, if you need to run it out, join the Karla Moore Memorial Tox 'n Purge 5K on Thursday morning!

Arch you glad you came to St. Louis? If the science alone isn't enough to convince you, the social events will! Join us of the Welcome Reception with the exhibitors on Tuesday evening, the Elmer Gordon Forum, and the Nite Owl Reception. The off-site event on Wednesday evening will take place at the historic Anheuser-Busch Brewery, for food and drinks, tours, live music from the Funky Butt Brass Band, and a meet-and-greet with a world-famous Budweiser Clydesdale. Thursday evening, we will celebrate President Tate Yeatman at the Skull and Crossbones Ball. Come dressed to impress with spooky flair and ready to dance to live music from the multi-piece band "Dirty Muggs".

Our "Cardinal" planning committee took our "Blues" away. We can't emphasize enough the outstanding effort of the SOFT 2024 planning committee and the SOFT Office. We thank each of you for your collaboration, knowledge, expertise, and guidance. And most importantly, we would like to thank all of you, the attendees, presenters, and exhibitors who make this meeting special every year.

All Aboard!
Sarah and Justin

PLANNING COMMITTEE

JUSTIN POKLIS

Host

SARAH RILEY

Host

SCIENTIFIC PROGRAM COORDINATORS
SARA DEMPSEY AND MATT JUHASCIK

WORKSHOP PROGRAM COORDINATORS
ALEX KROTULSKI AND VANESSA MENESES

FOOD & BEVERAGE COORDINATORS
ANN MARIE GORDON, DENICE TEEM, AND DELISA DOWNEY

MOBILE APPLICATION COORDINATORS
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VOLUNTEER COORDINATORS
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FUN RUN COORDINATOR
MADISON BEFFA AND KIMBERLY KARIN

AV COORDINATOR
FRANK WALLACE

YOUNG FORENSIC TOXICOLOGISTS
ERIN STRICKLAND

JAT SPECIAL ISSUE EDITOR
ERIN KARSCHNER

Sunday, October 27, 2024	Time	Place
Registration/Badge Pick Up	10am-5pm	Midway East
NSC-ADID Meeting	11am-3pm	Grand A
NLCP Inspector Training	2-6pm	Grand B/C
YFT Symposium	5-9pm	Grand E/F

Monday, October 28, 2024	Time	Place
Yoga	6-7am	Conductor Room
Registration/Badge Pick Up	7am-6pm	Midway East
Breakfast (WS Participants Only)	7-8:30am	Regency A/B
WS 1: Psychedelics	8am-12pm	Grand F
WS 2: Civil Cases	8am-12pm	Grand B/C
WS 3: Doping	8am-12pm	Grand A
WS 4: Impairment	8am-12pm	Grand D/E
ABFT Exam Committee	8am-5pm	Illinois
Break (WS Participants Only)	10-10:30am	Regency A/B
Waters Lunch and Learn	12-1:30pm	Midway 5
Agilent Lunch and Learn	12-1:30pm	Midway 6
Elmer Gordon Celebration Luncheon	12-1:30pm	Regency C
WS 5: ASB Standards	1:30-5:30pm	Grand B/C
WS 6: Breath Alcohol	1:30-5:30pm	Grand A
WS 7: Post Fentanyl Era	1:30-5:30pm	Grand F
Break (WS Participants Only)	3:30-4pm	Regency A/B
NPS Committee	5:30-6:30pm	New York
Drugs & Driving Committee	5:30-6:30pm	Midway 10

Tuesday, October 29, 2024	Time	Place
Yoga	6-7am	Conductor Room
Registration/Badge Pick Up	7am-6pm	Midway East
Breakfast (WS Participants Only)	7-8:30am	Regency A/B
AAFS Steering Committee	7-8:30am	New York
SOFT Board Meeting	8am-12pm	Missouri Pacific
WS 8: Lab Development	8am-12pm	Grand B/C
WS 9: Cannabinoids	8am-12pm	Grand F
WS 10: Oral Fluid	8am-12pm	Grand D/E
WS 11: ABFT Cert Pt 1	8am-12pm	Grand A
ABFT Exam	8am-12pm	Regency C
Break (WS Participants Only)	10-10:30am	Regency A/B
Sciex Lunch and Learn	12-1:30pm	Midway 5
Agilent Lunch and Learn	12-1:30pm	Midway 6
ThermoFisher Lunch and Learn	12-1:30pm	Midway 10
ABFT Board Meeting	12-5pm	Colorado Eagle
WS 12: ABFT Cert Pt 2	1:30-5:30pm	Grand B/C
WS 13: Sample Prep	1:30-5:30pm	Grand F
WS 14: LIMS	1:30-5:30pm	Grand D/E
WS 15: Diverse Orgs	1:30-5:30pm	Grand A
Break (WS Participants Only)	3:30-4pm	Regency A/B
Canadian Attendees	5:30-6:30pm	Red Cap Room
Oral Fluid Committee	5:30-6:30pm	New York
Publications Committee	5:30-6:30pm	Missouri Pacific
Postmortem Committee	5:30-6:30pm	Regency C

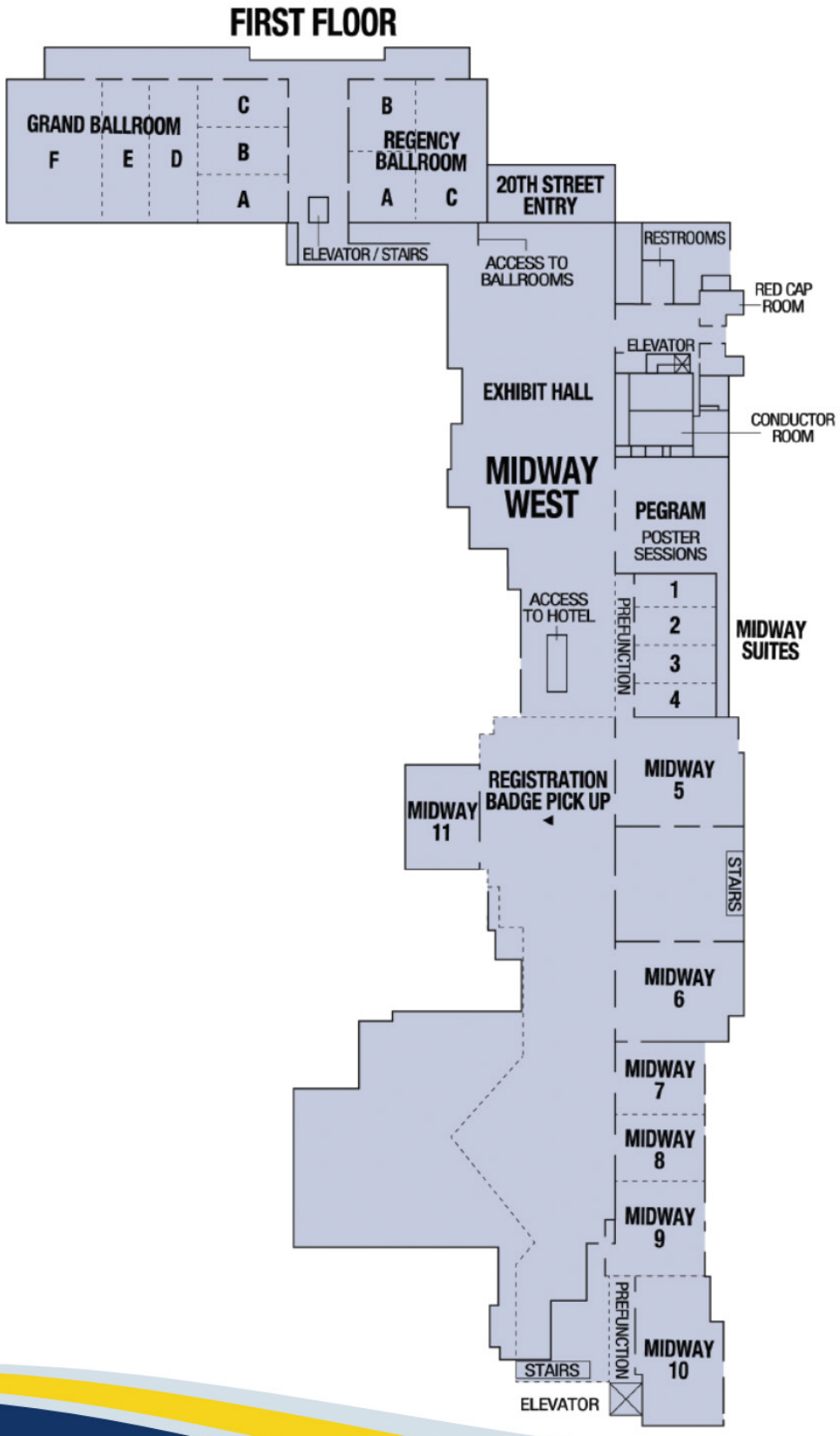
Tuesday, October 29, 2024	Time	Place
Welcome Reception	6:30-9:30pm	Midway West
Elmer Gordon Forum	8:30-10pm	Regency A/B/C
MilliporeSigma Nite Owl	10pm-12am	Grand Hall

Wednesday, October 30, 2024	Time	Place
Yoga	6-7am	Conductor Room
Registration/Badge Pick Up	7am-5pm	Midway East
Continental Breakfast	7-8:30am	Regency A/B
First-Time Attendee Breakfast	7-8am	Regency C
Poster Presentation Prep	7-11am	Pegram
Plenary Session	8-9am	Grand Ballroom
Session 1: Best Practices	9-10am	Grand Ballroom
Exhibit Hall	9:30am-4pm	Midway West
Refreshment Break	10-10:30am	Midway West
Session 2: Cannabis & Clinical	10:30am-12pm	Grand Ballroom
Lunch with Exhibitors	12-2pm	Midway West
Poster Session #1	12-2pm	Pegram
SOFT 2025 Planning Committee	12:30-1:30pm	Texas Special
Mentoring Program Lunch	12:30-1:30pm	Midway West
Session 3: Drugs and Driving	2-3:30pm	Grand Ballroom
Refreshment Break	3:30-4pm	Midway West
Session 4: Cannabis & Human Perf.	4-5pm	Grand Ballroom
Anheuser-Busch Brewery	6-10pm	20th Street Entry

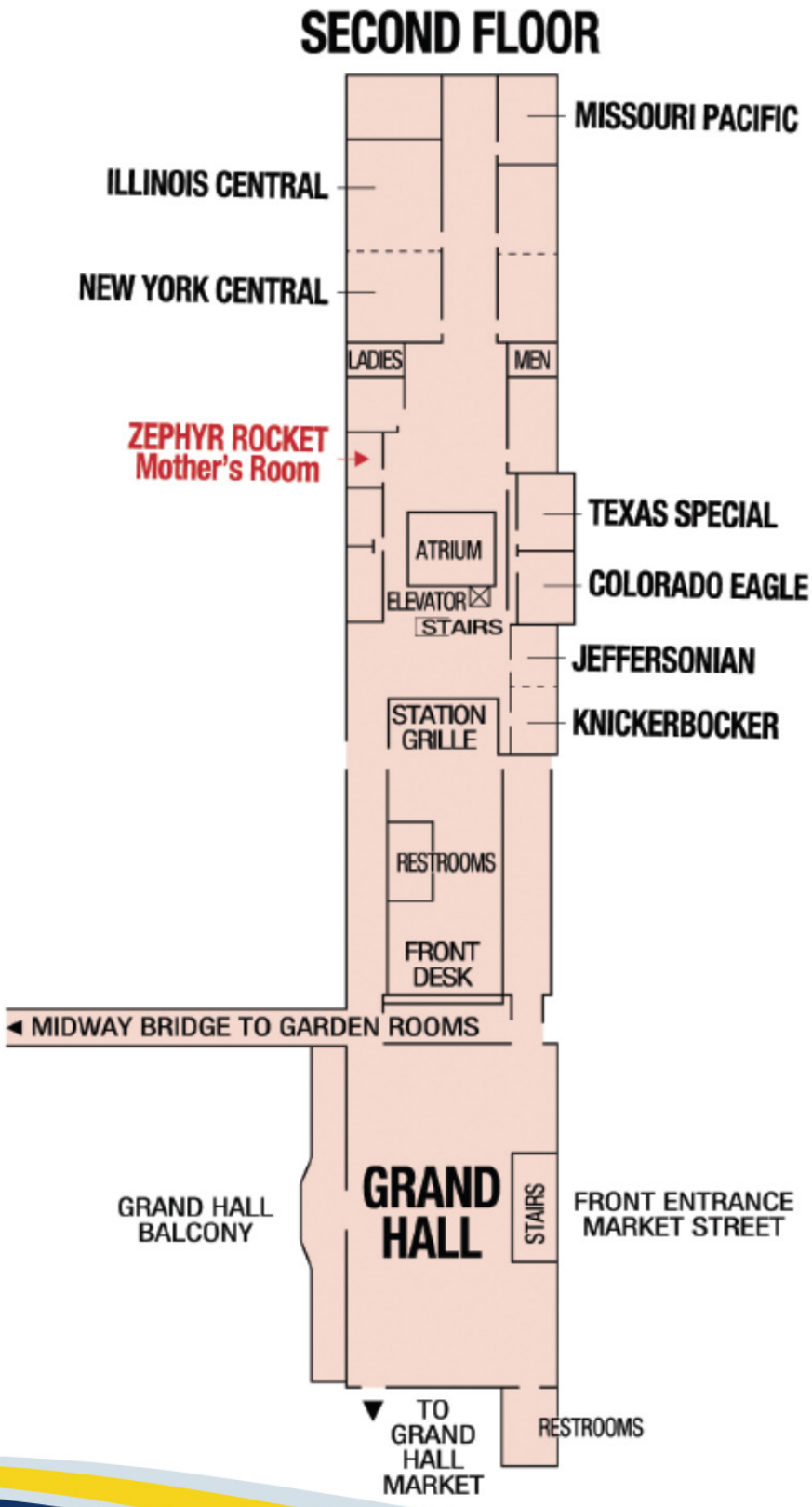
Thursday, October 31, 2024	Time	Place
Karla Moore Fun Run	6:30-8am	20th Street Entry
Registration/Badge Pick Up	7am-1pm	Midway East
Continental Breakfast	7-8:30am	Regency Ballroom
Poster Presentation Prep	7-11am	Pegram
Session 5: Analytical & PM	8-10am	Grand Ballroom
Exhibitor Feedback Meeting	8:30-9:30am	Midway 1/2
Exhibit Hall	9:30am-3:30pm	Midway West
Refreshment Break	10-10:30am	Midway West
Session 6: NPS & Analytical	10:30am-12pm	Grand Ballroom
Lunch with Exhibitors	12-2pm	Midway West
Poster Session #2	12-2pm	Pegram
DFC Committee	12:30-1:30pm	Midway 1/2
Session 7: Awards	2-3pm	Grand Ballroom
Refreshment Break	3-3:30pm	Midway West
SOFT Business Meeting	3:30-5pm	Grand F
Happy Hour	6-7pm	Foyer A/B/C
President's Banquet	7-8:30pm	Grand Ballroom
Live Band and Dancing	8:30pm-12am	Grand Ballroom

Friday, November 1, 2024	Time	Place
Continental Breakfast	7-8:30am	Regency Ballroom
Ses. 8: NPS, Cannabis & Alt. Matrices	8-10am	Grand Ballroom
Refreshment Break	10-10:30am	Regency Ballroom
Ses 9: Alt. Matrice, Analytical & PM	10:30am-12pm	Grand Ballroom

UNION STATION MAP



UNION STATION MAP



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HEAR FROM THE BOARD OF DIRECTORS AT THE SOFT BUSINESS MEETING ON THURSDAY, OCTOBER 31 FROM 3:30 - 5:00 PM

- OFFICER REPORTS
- AWARDS
- FREE REGISTRATION RAFFLE

THANK YOU VOLUNTEERS!

Thank you to all the incredible volunteers who have generously dedicated their time, expertise, and energy into making this SOFT meeting a success! Your commitment and hard work have truly made a difference—from behind-the-scenes support to direct interactions with our attendees. This meeting wouldn't have been possible without your efforts, enthusiasm, and dedication. We deeply appreciate everything you've done to ensure a smooth and memorable experience for everyone. Thank you for being an invaluable part of SOFT!

WORKSHOP PROGRAM CHAIRS

Workshop 1: Kristin Kahl & Michael A. Wagner
Workshop 2: Madeleine Swortwood & Curt Harper
Workshop 3: Pascal Kintz & Anne-Laure Pelissier
Workshop 4: Jasmine Maxwell & Suman Rana
Workshop 5: Robert Johnson & Kei Osawa
Workshop 6: Nita Bolz & Jasmine Maxwell
Workshop 7: Sara Walton & Marthe Vandeputte
Workshop 8: Luke Rodda & Dani Mata
Workshop 9: Szabolcs Sofalvi & Svante Vikingsson
Workshop 10: Amanda Mohr & Nathalie Desrosiers
Workshop 11/12: Marissa Finkelstein & Erika Phung
Workshop 13: Victor Vandell & Sarah Bartock
Workshop 14: Hunter Fleming & Allie Mennella
Workshop 15: Oliver Grundmann & Samantha Tolliver

THANK YOU VOLUNTEERS!

SCIENTIFIC SESSION MODERATORS

Session 1: Sarah Riley & Justin Poklis
Session 2: Lindsay Glicksberg & Sara Short
Session 3: Erin Karschner & Dayong Lee
Session 4: Joe Kahl & Kim Samano
Session 5: Dan Anderson & Jessica Gleba
Session 6: Nick Tiscione & Rebecca Wagner
Session 7: Sara Schreiber & Tim Rohrig
Session 8: Teresa Gray & Kayla Neuman
Session 9: Sara Dempsey & Matthew Juhascik
Poster Session 1: Melissa Rodriguez & Brianna Peterson
Poster Session 2: Erin Jeffrey & Kristin Kahl

ABSTRACT REVIEWERS

Afton Martinez
Alaina Holt
Alberto Salomone
Alexander San Nicolas
Allen Gilliland
Alli Timmons
Amanda Cadau
Amanda Jenkins
Amanda Pacana
Andrea Fox
Anisha Paul
Ann-Sophie Korb
Aracelis Angelica Velez
Austin Ciesielski
Ayako Chan-Hosokawa
Belicia Sutton
Blake Stutzman
Britni Skillman
Carl Wolf
Carrie Kirkton
Carrol Nanco
Christina Smith
Christopher Cording
Christopher Dal Chele
Colleen Moore
Conne Lewis
Curtis Oleschuk
Dan Anderson
Dani Mata
Daniel Baker
David Eagertonn
Delisa Downey
Denice Teem

THANK YOU VOLUNTEERS!

ABSTRACT REVIEWERS

Douglas Rohde
Elisa Shoff
Erika Phung
Erin Hensel
Erin Karschner
Erin Spargo
Erin Strickland
Gail Cooper
Garry Milman
Gary Kunsman
Haley Mulder
Harry Qui
Heather Barkholtz
Heather Ciallella
Irene Shu
James Fleming
James Kraner
Janet Putnam
Jared Castellani
Jay Gehlhausen
Jeff Hackett
Jeff Walterscheid
Jennifer Hobbs
Jennifer Swatek
Jeremy Smith
Jeri Ropero-Miller
Jessi Dyck
Jillian Neifeld
Jocelyn Abonamah
Jochen Beyer
Joe Kahl
John Kucmanic
Jon Stephenson
Jonathan Tomko
Joseph Saady
Julia Pearson
Justin Poklis
Kaitlyn Palmquist-Orlando
Karen Scott
Kari Midthun
Katherine Bollinger
Katherine Ridinger
Kayla Ellefsen
Kayla Neuman
Kayla Smith
Kelly Maychack
Kenneth Ferslew
Kim Samano
Kimberle Glowacki
Kimberley Heine
Kristen Steward
Kristin Kahl
Laerissa Reveil
Laureen Marinetti
Leanne Hazard
Lee Blum
Lindsay Glicksberg
Lixia Chen
Luigino Apollonio
Madeleine Wood
Madeline Montgomery
Mandi Mohr
Manoj Tyagi
Maria A. Martinez
Maria Olds
Marilyn Huestis
Marissa Finkelstein
Mark Villoria
Marta Concheiro-Guisan
Mary Jones
Matthew Juhascik
Meaghan Hessler

THANK YOU VOLUNTEERS!

ABSTRACT REVIEWERS

Megan Barton
Melissa Beals
Melissa Fogarty
Melissa Lloyd
Melissa Rodriguez
Michael Chen
Michael Fagiola
Michael Frontz
Michael Smith
Michael Stypa
Michael Truver
Michael Wagner
Michelle Carlin
Nikolas Lemos
Oliver Grundmann
Peter Koin
Peter Maskell
Philip Kemp
Prenstiss Jones
Pucheng Ke
Rachel Barnett
Rachel Marvin
Rebecca Hartman
Rebecca Phipps
Rebecca Wagner
Robert Almeida
Robert Johnson
Robert Sears
Roberta Gorziza
Robert Kronstrand
Samuel Kleinman
Sara Dempsey
Sara Schreiber
Sara Walton
Sarah Buxton de Quintana
Sarah Riley
Scott Larson
Stephanie Marco
Stephanie Marin
Stephen Raso
Sue Pearring
Sumandeep Rana
Sunday Hickerson
Tate Yeatman
Teresa Gray
Thomas Keller
Tiffany Moreno
Tim Rohrig
Tyler Devincenzi
Tyson Baird
Uttam Garg
Vanessa Meneses
Veronica Hargrove
Wen Dui
William Dunn
Zhenqian Zhu

THANK YOU VOLUNTEERS!

JAT SPECIAL ISSUE REVIEWERS

Aaron Shapiro
Alberto Salomone
Alex Krotulksi
Amanda Mohr
Amy Patton
Brittany Casey
Carl Wolf
Colleen Moore
Curt Harper
Dan Anderson
Elisa Shoff
Gail Cooper
Haley Mulder
Heather Ciallella
Jarrad Wagner
Jason Hudson
Jeff Walterscheid
Jessica Ayala
Jolene Bierly
Joseph Kahl
Joshua Seither
Julia Pearson
Justin Brower
Justin Poklis
Kaitlyn Palmquist
Kayla Ellefsen
Kevin Shanks
Laura Labay
Luke Rodda
Madeleine Wood
Matt Juhascik
Melissa Fogarty
Michael Truver
Nathalie Desrosiers
Peter Stout
Rebecca Boswell
Rebecca Hartman
Rebecca Wagner
Robert Johnson
Robert Kronstrand
Sandra Bishop-Freeman
Sara Dempsey
Sara Walton
Sarah Bartock
Svante Vikingsson
Veronica Hargrove

THANK YOU SPONSORS!

TIER I SPONSOR - $15,500

SOFT Events Sponsored by Tier I Sponsors Include:
Welcome Reception, Elmer Gordon Forum & Dessert Reception, President Yeatman's Skull & Bones Cocktail Hour & Banquet Reception, Continental Breakfasts, Meeting Luncheons, Refreshment Breaks

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SHIMADZU
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UCT

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SOFT Events Sponsored by Tier II Sponsors Include:
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TIER III SPONSOR - $3,500

SOFT Events Sponsored by Tier III Sponsors Include:
Continental Breakfasts, Meeting Luncheons & Refreshment Breaks

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TIER IV SPONSORSHIP - $1,500

SOFT Events Sponsored by Tier IV Sponsors Include:
Continental Breakfasts & Refreshment Breaks

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TIER V SPONSORSHIP - $1,000

SOFT Events Sponsored by Tier V Sponsors Include:
Continental Breakfasts

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THANK YOU SPONSORS!

KARLA MOORE FUN RUN/WALK SPONSOR - $250

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KARLA MOORE FUN RUN/WALK
THURSDAY OCTOBER 31 6:30-8:00 AM

About the Fun Run/Walk

Join us for the Karla Moore Fun Run/Walk bright and early on **Thursday, October 31 at 6:30 am**! Participants may run or walk the route. Volunteers with signage will be along the route to help direct you. Prizes are not provided for winners but you get bragging rights! **Your $30 donation includes a Fun Run shirt & lapel pin. Proceeds are donated to the American Cancer Society in Dr. Moore's memory.**

Fun Run/Walk History

The original Tox 'N Purge run was **created by Dr. Karla Moore in 1997 for the Salt Lake City meeting**. In addition to her 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 and expenses for the event are supported by our SOFT exhibitors.

NETWORKING OPPORTUNITIES

MORNING YOGA

Monday, October 28 - Tuesday, October 29 - Wednesday, October 31
Time: 6:00 - 7:00 am
We're excited to host morning yoga sessions at this year's SOFT Meeting! Join us Monday, Tuesday, and Wednesday from 6-7 AM to start your day with relaxation and rejuvenation. Whether you're a seasoned yogi or new to the practice, all are welcome to participate. Equipment is provided.

WELCOME RECEPTION

Tuesday, October 29
Time: 6:30 - 9:30 pm
Join us for the grand opening of SOFT's Exhibit Hall! Explore the exhibits, reconnect with colleagues, and enjoy the provided food and beverages. Don't forget to check out the Exhibit Hall Map in SOFT's Mobile App. This event is open to all SOFT attendees.

ELMER GORDON FORUM DESSERT RECEPTION

Tuesday, October 29
Time: 8:30 - 10:00 pm
SOFTopics and the Elmer Gordon Forum are teaming up again this year at the 2024 SOFT Meeting in St. Louis, MO! Join us and meet some of the people you've gotten to know over our virtual discussions. Don't be shy in asking your fellow toxicologists in the room your burning questions or share interesting observations from your laboratory – when do you have so many toxicologists in one room?! This event is open to all SOFT attendees.

MILLIPORESIGMA NITE OWL RECEPTION

Tuesday, October 29
Time: 10:00 pm - 12:00 am
End your evening at the Nite Owl Reception, hosted by our Tier II sponsor, MilliporeSigma. This event is open to all SOFT attendees.

NETWORKING OPPORTUNITIES

FIRST TIME ATTENDEE BREAKFAST

Wednesday, October 30
Time: 7:00 - 8:00 am
Is this your first time attending a SOFT meeting? Fantastic! Don't miss the chance to connect with the SOFT Board of Directors and fellow newcomers at the First-Time Attendee Breakfast. This event is open to all First Time Attendees!

ANHEUSER BUSCH BREWERY TOUR

Wednesday, October 30
Time: 6:00 - 10:00 pm
Don't miss this exciting off-site event! Transportation will be provided to and from the venue, food and beverages will be available on-site. Meet one of the world-famous Clydesdales! This event is open to all SOFT attendees.

KARLA MOORE FUN RUN/WALK

Thursday, October 31
Time: 6:30 - 8:00 am
Join us for the Karla Moore Fun Run/Walk, a tradition that began in 1997 with the Tox 'N Purge run, created by Dr. Karla Moore. Proceeds from this event will be donated to the American Cancer Society in her memory. Donation: $30 (includes a lapel pin & shirt)

PRESIDENT YEATMAN'S SKULL & CROSSBONES BALL

Thursday, October 31
Cocktail Hour: 6:00 - 7:00 pm
Dinner and Dancing: 7:00 pm - 12:00 am
Enjoy an elegant evening of dinner, drinks, and dancing. We encourage attendees to Dress to Impress as we celebrate together! Cocktail attire is suggested with a playful emphasis on skulls, bones and Halloween.

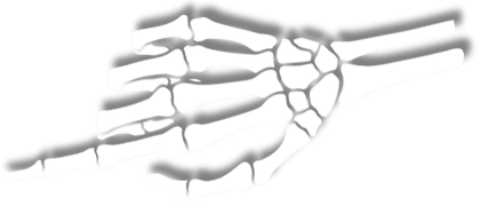
YFT SYMPOSIUM & PROFESSIONAL DEVELOPMENT FAIR

Sunday, October 27, 5:00 - 9:00 pm

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

Symposium Panel Speakers:

- Dr. Teresa Gray – Certification/Professional Organization
- Dr. Nathalie Desrosiers - Public Lab
- Andre Sukta - Research & Development
- Dr. Alex Krotulski - Higher Education
- Dr. Jennifer Colby – Private Lab



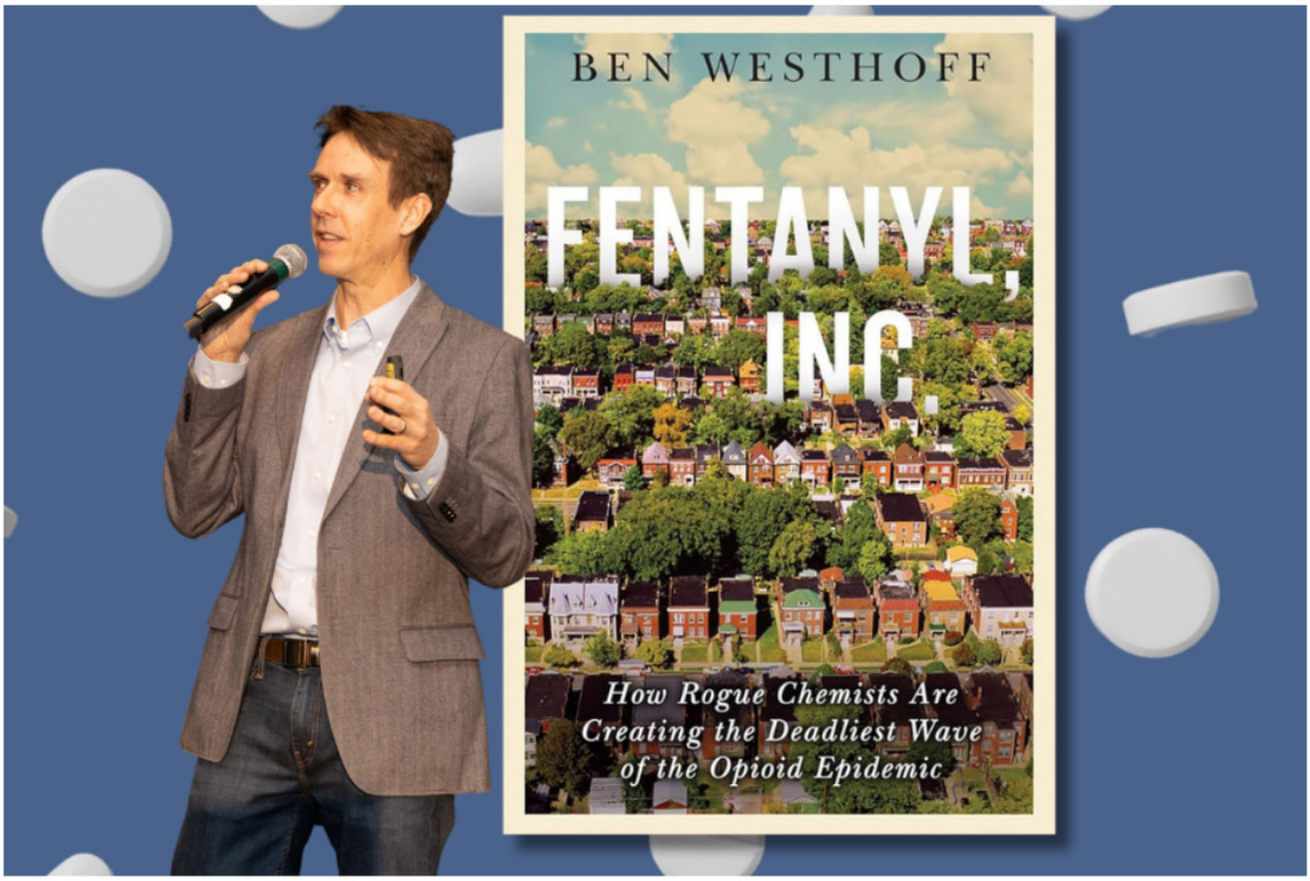
SUBMIT A QUESTION FOR THE PANEL SPEAKERS!

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

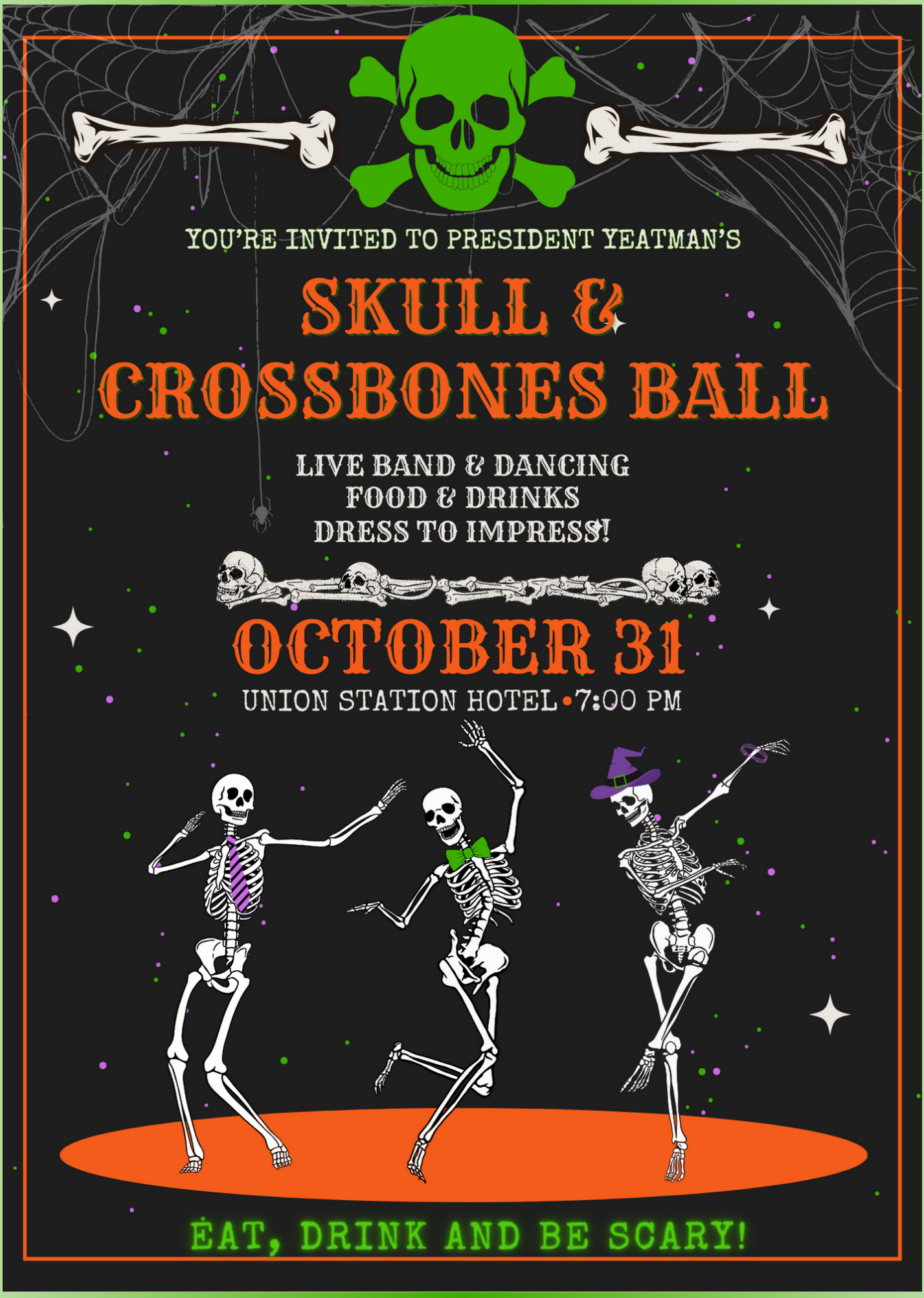
HOSTED BY THE YOUNG FORENSIC TOXICOLOGISTS COMMITTEE

PLENARY SPEAKER BEN WESTHOFF

Wednesday, October 30
8:00 - 9:00 am



We are thrilled to welcome **Ben Westhoff** as the Plenary Speaker for this year's meeting! Ben is the author of the bestselling nonfiction book **Fentanyl Inc: How Rogue Chemists are Creating the Deadliest Wave of the Opioid Crisis**, which was named a Telegraph Book of the Year. As an investigative journalist, Ben conducted an undercover investigation into the synthetic-drug epidemic traveling to China to meet with clandestine chemists supplying precursor materials to illicit fentanyl manufacturers. He will be sharing his insights and experiences with us.


YOU'RE INVITED TO PRESIDENT YEATMAN'S
SKULL & CROSSBONES BALL
LIVE BAND & DANCING
FOOD & DRINKS
DRESS TO IMPRESS!
OCTOBER 31
UNION STATION HOTEL • 7:00 PM
EAT, DRINK AND BE SCARY!

WHAT TO EXPECT AT THE SKULL & CROSSBONES BALL

SUGGESTED ATTIRE

We encourage SOFT attendees to DRESS TO IMPRESS as we celebrate together! Cocktail attire is suggested with a playful emphasis on skulls, bones, and Halloween. Examples are included below:

EAT, DRINK AND BE SCARY!

WORKSHOP PROGRAM

PROGRAM COORDINATORS: ALEX KROTULSKI & VANESSA MENESES

MONDAY, OCTOBER 28

MORNING: 8:00 AM -12:00 PM

Workshop 1: Psychedelics: Promising Treatment Applications and Concerns for Impaired Driving
Chairs: Kristin Kahl & Michael Wagner
Audience: Basic

Workshop 2: More Likely Than Not: Navigating Civil Cases in Forensic Toxicology
Chairs: Madeleine Swortwood & Curt Harper
Audience: Basic

Workshop 3: Forensic Aspects of Doping
Chairs: Pascal Kintz & Anne-Laure Pélissier
Audience: Intermediate

Workshop 4: Chasing Impairment – Advancement in Technologies or a Paradigm Shift? – You be the Judge!
Chairs: Suman Rana & Jasmine Maxwell
Audience: Intermediate

AFTERNOON: 1:30 - 5:30 PM

Workshop 5: ASB Standards: Overview, Implementation, and Updates
Chairs: Robert Johnson & Kei Osawa
Audience: Basic

Workshop 6: Breath Alcohol Testing Program Operation and Management
Chairs: Nita Bolz & Jasmine Maxwell
Audience: Basic

Workshop 7: Are we Entering the Post-fentanyl Era – Or Have We Already Entered It?
Chairs: Marthe Vandeputte & Sara Walton
Audience: Intermediate

WORKSHOP PROGRAM

TUESDAY, OCTOBER 29

MORNING: 8:00 AM -12:00 PM

Workshop 8: Laboratory Development: Where to Start and How to Continually Improve
Chairs: Dani Mata & Luke Rodda
Audience: Intermediate

Workshop 9: Recent Research on the Semi-synthetic Cannabinoids Δ8-THC, THCO, and HHC
Chairs: Szabolcs Sofalvi & Svante Vikingsson
Audience: Intermediate

Workshop 10: Presenting Oral Fluid Drug Testing Results in Court
Chairs: Amanda Mohr & Nathalie Desrosiers
Audience: Intermediate

Workshop 11: The ABCs of ABFT Certification – Part I: General Information
Chairs: Marissa Finkelstein & Erika Phung
Audience: Basic

AFTERNOON: 1:30 - 5:30 PM

Workshop 12: The ABCs of ABFT Certification – Part II: Study Topics in Forensic Toxicology
Chairs: Marissa Finkelstein & Erika Phung
Audience: Intermediate

Workshop 13: How Am I Suppose To Analyze That?! A Master Class in Sample Preparation
Chairs: Victor Vandell & Sarah Bartock
Audience: Intermediate

Workshop 14: Laboratory Information Management Systems (LIMS): Streamlining Forensic Toxicology Workflows
Chairs: Hunter Fleming & Allie Mannella
Audience: Intermediate

Workshop 15: Come as You are: Building a Diverse and Accessible Organization
Chairs: Oliver Grundmann & Samantha Tolliver
Audience: Basic

SCIENTIFIC PROGRAM

PROGRAM COORDINATORS: SARA DEMPSEY & MATT JUHASCIK

PRESENTATION LOCATION

- Platform Presentations: Grand Ballroom
- Poster Presentations: Pegram/Exhibit Hall

WEDNESDAY, OCTOBER 30

- Poster Preparation: 7:00 - 11:00 AM
- Platform Presentations: 9:00 AM - 5:00 PM
- Poster Session #1: 12:00 - 2:00 PM

THURSDAY, OCTOBER 31

- Poster Preparation: 7:00 - 11:00 AM
- Platform Presentations: 8:00 AM - 3:00 PM
- Poster Session #2: 12:00 - 2:00 PM

FRIDAY, NOVEMBER 1

- Platform Presentations: 8:00 AM - 12:00 PM

PLATFORM SESSION TOPICS

Session 1: Best Practices & Awards
Session 2: Cannabis & Clinical
Session 3: Drugs & Driving Special Session
Session 4: Human Performance & Cannabis
Session 5: Analytical Methods & Postmortem
Session 6: Novel Psychoactive Substances & Analytical Methods
Session 7: Awards & Best Practices
Session 8: Novel Psychoactive Substances, Cannabis, & Alternative Matrices
Session 9: Alternative Matrices, Analytical Methods, and Postmortem

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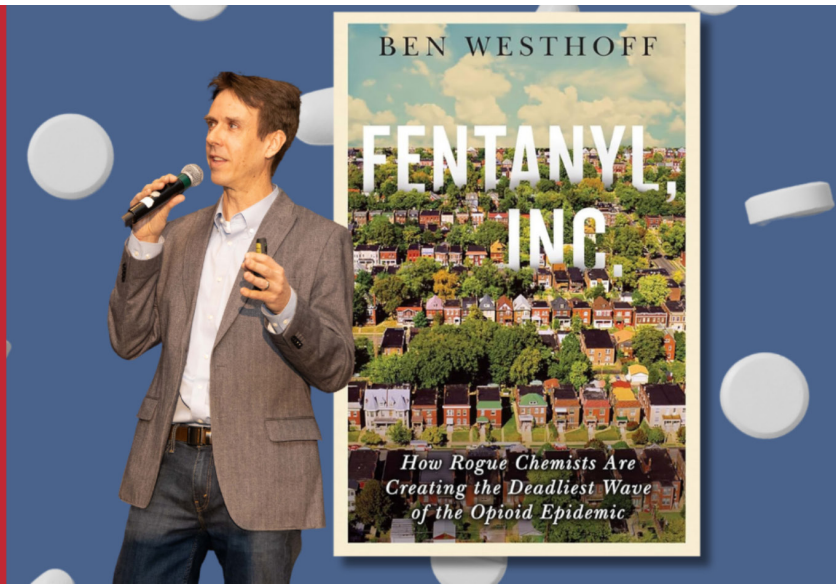
PLENARY SESSION
WEDNESDAY, OCTOBER 30
8:00-9:00 AM

SPEAKER: BEN WESTHOFF

FENTANYL INC: HOW ROGUE CHEMISTS ARE CREATING THE DEADLIEST WAVE OF THE OPIOID CRISIS



SESSION 1
WEDNESDAY, OCTOBER 30
9:00-10:00 AM
MODERATORS: SARAH RILEY & JUSTIN POKLIS
TOPIC: BEST PRACTICES & AWARDS

TIME	#	TITLE	SPEAKER
9:00-9:12 am	S1	Successful application of a prediction model for the direct alcohol biomarker phosphatidylethanol 16:0/18:1 (PEth) in a judicial context	Christophe P Stove
9:12-9:24 am	S2	Addressing QC Tracking and Measurement Uncertainty With Simple Excel Spreadsheets, Without Automation or Macros	Rebecca L. Hartman
9:24-9:36 am	S3	International Cooperation Supporting the Development of Forensic Science	Mark Mogle
9:36-9:48 am	S4	Rebranding the Postmortem Toxicology Laboratory: Creating New Partnerships within Public Health	Sandra Bishop-Freeman
9:48-10:00 am	S5	Nitazenes of the past, present, and future: Insights from in vitro μ-opioid receptor assays and in vivo behavioral studies in mice	Marthe Vandeputte
10:00-10:30 AM - MORNING BREAK			

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SESSION 2 WEDNESDAY, OCTOBER 30 10:30 AM - 12:00 PM MODERATORS: LINDSAY GLICKSBERG & SARA SHORT TOPICS: CANNABIS AND CLINICAL			
Time	**#**	**TITLE**	**SPEAKER**
10:30-10:42 am	S6	In vitro metabolism of Δ8-tetrahydrocannabinol (Δ8-THC) and Δ8-THC-O acetate (Δ8-THCO)	Ya-Chih Cheng
10:42-10:54 am	S7	The Prevalence of Delta-8-THC in Orange County, CA	Melanie Hernandez
10:54-11:06 am	S8	Non-fatal overdose bio-surveillance: A multi-site cross-sectional pilot study	Heather Barkholtz
11:06-11:18 am	S9	Urinary Fentanyl Concentrations in Patients Dosed with Fentanyl from Transdermal Patches, Tablets, Lozenges, or Sublingual Spray	Aron Jaffe
11:18-11:30 am	S10	Adverse events and mitragynine and 7-hydroxymitragynine plasma pharmacokinetics after controlled mitragynine isolate to healthy human participants	Marilyn Huestis
11:30-11:42 am	S11	Quantitative Analysis of Traditional Drugs and NPS in Blood Specimens Collected from Emergency Department Patients After Suspected Drug Overdose	Devin Kress
11:42-11:54 am	S12	Drugs of abuse influence inflammation profiles in deceased and living individuals	Matthew Levitas
12:00-2:00 PM - LUNCH			

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SESSION 3 WEDNESDAY, OCTOBER 30 2:00-3:30 PM MODERATORS: ERIN KARSCHNER & DAYONG LEE TOPIC: DRUGS AND DRIVING SPECIAL SESSION			
Time	**#**	**TITLE**	**SPEAKER**
2:00-2:12 pm	S13	New York State Police's Evidential Oral Fluid Testing Pilot Program	Jennifer F. Limoges
2:12-2:24 pm	S14	Changes in blood cannabinoid concentrations over multiple collection times in driving under the influence of drug(s) casework	Brianna Peterson
2:24-2:36 pm	S15	A Ketamine Trilogy: A Review of Three Different DUID Cases with the Same Driver.	Edward Zumaeta
2:36-2:48 pm	S16	Results from the 2024 Survey for Drug Testing in DUID and Motor Vehicle Fatality Investigations	Amanda D'Orazio
2:48-3:00 pm	S17	Driving under influence of delta8-tetrahydrocanabinol cases in Houston, Texas	Ashley Ann Johnson
3:00-3:12 pm	S18	Evaluation of Securetec DrugWipe® S as an Approved Roadside Oral Fluid Device in Alabama	Kristin Umstead
3:12-3:24 pm	S19	Application of LC-HRMS Screening to Blood DUID Cases: Benefits & Limitations	Nicholas Tiscione
3:30-4:00 PM - AFTERNOON BREAK			

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SESSION 4
WEDNESDAY, OCTOBER 30
4:00-5:00 PM
MODERATORS: JOE KAHL & KIM SAMANO
TOPICS: HUMAN PERFORMANCE AND CANNABIS

Time	#	TITLE	SPEAKER
4:00-4:12 pm	S20	Pulse testing of negative federally regulated urine specimens by LC-QTOF-MS indicates use of additional impairing substances	Olivia Skirnick
4:12-4:24 pm	S21	Evaluation of Sevoflurane Interference with Alcohol Reporting, Including a Potential Authentic Case Sample	Charles Perkins
4:24-4:36 pm	S22	Current trends in psilocin casework	Sarah Doumit
4:36-4:48 pm	S23	Analysis of Nutraceutical Products for Pharmacologically Active Substances	Lillie Thomas
4:48-5:00 pm	S24	Analysis of Vaping Products Confiscated from Public School Systems Spanning Across the Commonwealth of Virginia	Kelsey Moss

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SESSION 5 THURSDAY, OCTOBER 31 8:00-10:00 AM MODERATORS: DAN ANDERSON & JESSICA GLEBA TOPICS: ANALYTICAL METHODS AND POSTMORTEM			
Time	**#**	**TITLE**	**SPEAKER**
8:00-8:12 am	S25	A Novel Screening Workflow for Nitazene Analogs using LC-QQQ Precursor Ion Scan Acquisition	Amanda Pacana
8:12-8:24 am	S26	Evaluation into Action - Validation of a Comprehensive 48-well Plate SLE Quantitative Method for all Tier I Compounds (and more) via LC/MS-MS after Laboratory Evaluation Using NHTSA's DUID tool.	Jarrod Bechard
8:24-8:36 am	S27	Interference of exo-THC with delta9-THC in LC-MS/MS confirmation analysis	Erin Cosme
8:36-8:48 am	S28	Development and Validation of a Screening and Confirmation Method of 947 Substances by LC-QTOF-MS for Routine Application in Forensic Toxicology Casework	Maria Sarkisian
8:48-9:00 am	S29	Automated and rapid analysis of over 200 drugs in blood and urine for DUID, DFC, and postmortem investigations	Steven Towler
9:00-9:12 am	S30	Update: OSAC Forensic Toxicology Subcommittee and the Development of a Standard Practice and Standard Test Method for the Analysis of Volatiles	Rebecca Wagner
9:12-9:24 am	S31	The Dark Side of the SHROOM: A Retrospective Study of Psilocin Identified in Post-mortem Cases in Miami-Dade County, Florida	Jose Garcia
9:24-9:36 am	S32	Postmortem forensic toxicology interpretation: a likelihood ratio approach	Brigitte Desharnais
9:36-9:48 am	S33	Opioids in Overdose Cases in Alabama (2022-2024)	Keeyahna Foster
9:48-10:00 am	S34	Tizanidine: The Other Alpha-2 Adrenergic Agonist	Laura Friederich
10:00-10:30 AM - MORNING BREAK			

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SESSION 6

THURSDAY, OCTOBER 31

10:30 AM - 12:00 PM

MODERATORS: NICK TISCIONE AND REBECCA WAGNER

TOPICS: NOVEL PSYCHOACTIVE SUBSTANCES AND ANALYTICAL METHODS

Time	#	TITLE	SPEAKER
10:30-10:42 am	S35	A 5 Year Review of Postmortem NPS Trends in Travis County, Texas	Kayla N. Ellefsen
10:42-10:54 am	S36	A “Tail” of Two Cities – When Old Synthetic Cannabinoids Become New Again	Alex Krotulski
10:54-11:06 am	S37	Trends in Tranq: Prevalence of Xylazine in Oral Fluid Toxicology in Michigan, Ohio and Indiana	Chris Thomas
11:06-11:18 am	S38	Characterization of Reactive Metabolites for Eight NPS Using Glutathione and N-Acetylcysteine Trapping Assays	Leonardo Maya
11:18-11:30 am	S39	Novel Psychoactive Substances: Comparative Performance of LC-QTOF-MS Acquisition Methods for Nontargeted Analysis in Urine and Whole Blood	Akshita Verma
11:30-11:42 am	S40	Pharmacological characterization and forensic case series of emerging 2-benzylbenzimidazole ‘nitazene’ opioids	Liam M. De Vrieze
11:42-11:54 am	S41	The History of Breath Alcohol Detection	M Rankine Forrester Jr
12:00-2:00 PM - LUNCH			

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SESSION 7
THURSDAY, OCTOBER 31
2:00-3:30 PM
MODERATORS: SARA SCHREIBER & TIM ROHRIG
TOPICS: AWARDS AND BEST PRACTICES

Time	#	TITLE	SPEAKER
2:00-2:12 pm	S42	Rate of Positive Drug Results Following the ASB's Minimum Testing Standards in Postmortem and Impaired Driver Cases	Brian Simpson
2:12-2:24 pm	S43	Analysis of 28 Phytocannabinoids and Semi-Synthetic Cannabinoids in Blood and Urine by LC-MS/MS	Luette Muir
2:24-2:36 pm	S44	Comparison of Ketone Levels Using a Commercial Ketone Monitoring System to Screen for Beta-hydroxybutyrate Prior to GC-MS Analysis.	Kerry Murr
2:36-2:48 pm	S45	Determining Cannabinoid Acetate Analog (Δ9-THC-O-A, Δ8-THC-O-A, CBD-di-O-A) Metabolites Using Human Liver Microsomes to Determine the Structure and Relative Appearance of Metabolism	Natalie Ortiz
2:48-3:00 pm	S46	2024 Update on Standards Development Activities in Forensic Toxicology	Marc LeBeau
3:00-3:30 PM - AFTERNOON BREAK			

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SESSION 8 FRIDAY, NOVEMBER 1 8:00-10:00 AM MODERATORS: TERESA GRAY & KAYLA NEUMAN TOPICS: NOVEL PSYCHOACTIVE SUBSTANCES, CANNABIS, AND ALTERNATIVE MATRICES			
Time	**#**	**TITLE**	**SPEAKER**
8:00-8:12 am	S47	Evaluation of a Case Series Involving N,N-Dimethylpentylone: A Retrospective Study and Trends	Marco Ballotari
8:12-8:24 am	S48	Emergence of ortho-Methylfentanyl in Medicolegal Death Investigation Cases from North America	Sara Walton
8:24-8:36 am	S49	Optimization of generative AI tools for high throughput detection of NPS by retrospective analysis of HRMS data	Aaron M. Shapiro
8:36-8:48 am	S50	Emergence of Medetomidine as an Opioid Adulterant Encountered with Fentanyl, Xylazine, and Other Substances	Barry Logan
8:48-9:00 am	S51	Prevalence of Psychiatric Medication Use Among Pilots Involved in General Aviation Accidents from FY2019 to FY2023	Kimberly Karin
9:00-9:12 am	S52	Update on Acute and Chronic Oral Dosing of Cannabidiol (CBD) With and Without Low Doses of Delta-9-Tetrahydrocannabinol (Δ9-THC)	Svante Vikingsson
9:12-9:24 am	S53	Hemp-derived or semisynthetic cannabinoids: pharmacology of isomers and how it matters for the harm potential of seized drugs	Christophe P Stove
9:24-9:36 am	S54	Investigation into CBD user urine testing positive for THC in a drug screening kit	Maiko Kusano
9:36-9:48 am	S55	In Vitro Formation of 6-Monoacetylmorphine (6MAM) in Oral Fluid Post Collection in the Presence of Morphine and Aspirin	Omran Muslin
9:48-10:00 am	S56	Synthetic Stimulant of the Southeast – Evaluating Continued Prevalence of N,N-Dimethylpentylone	Michael Lamb
10:00-10:30 AM - MORNING BREAK			

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SESSION 9

FRIDAY, NOVEMBER 1

10:30 AM - 12:00 PM

MODERATORS: SARA DEMPSEY & MATTHEW JUHASCIK

TOPICS: ALTERNATIVE MATRICES, ANALYTICAL METHODS, AND POSTMORTEM

Time	#	TITLE	SPEAKER
10:30-10:42 am	S57	Development of a Semi-Quantitative Liquid Chromatography Tandem Mass Spectrometry Method for 62 Drugs in Umbilical Cord Tissue	Tiara Evans
10:42-10:54 am	S58	Rapid Quantitative Screening of 16 Synthetic Cannabinoids in Urine Using DART-MS Analysis	Terry Bates
10:54-11:06 am	S59	Determination of Drugs and Metabolites in Oral Fluid: Comparison of Different Extraction Procedures	Marta Concheiro-Guisan
11:06-11:18 am	S60	Prevalence and Analysis of Xylazine in Postmortem Casework in Jefferson County, Alabama	Karen S Scott
11:18-11:30 am	S61	Monitoring Drug Overdose Death in the United States, 1999-2023	Margaret Warner
11:30-11:42 am	S62	Interpretation of prescription drug concentrations in opioid-related drug deaths. Should these drugs be included on the death certificate?	Craig Chatterton
11:42-11:54 am	S63	Toxicology Findings in Train-Related Pedestrian Fatalities in Harris County, Texas	Teresa Gray

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POSTER SESSION #1
WEDNESDAY, OCTOBER 30, 12:00-2:00 PM
MODERATORS: MELISSA RODRIGUEZ AND BRIANNA PETERSON

#	TITLE	SPEAKER
P1	An Update to Human Red Blood Cell Acetylcholinesterase Activity Reference Ranges for Acute Organophosphate Poisoning Detection	Pucheng Ke
P2	Detection of Xylazine in Whole Blood Samples Using High-Resolution Mass Spectrometry	Karl Oetjen
P3	Detection of Nitazenes in Vape Juice	Pierre Negri
P4	Withdrawn	
P5	Withdrawn	
P6	Δ-8- and Δ-9-THC-Carboxy Metabolites in Urine Drug Testing Specimens at CRL from April 2023 to January 2024	David Kuntz
P7	Trends in Fentanyl Analysis in Workplace Urine Drug Testing at CRL	Melissa Beals
P8	CRL Testing Experience for Fentanyl in Oral Fluid	Michael Clark
P9	Quantitation of an Oral Fluid Drug Panel Including THC Using High Resolution Accurate-Mass (HRAM) Orbitrap Mass Spectrometry	Courtney Patterson
P10	A Fully Validated LC-QTOF-MS/MS Screening Workflow for the Analysis of Drugs in Oral Fluid	Cindy Coulter
P11	Comparison of Return on Investment of a LC-QTOF Screening Method and an ELISA Screening Method	Samantha Wong
P12	Nitazene analog detection and involvement in drug overdose deaths — United States, 2019–2022	Maria Zlotorzynska
P13	Occurrence of Opioids in Forensic Casework in the State of Sergipe in Brazil (2019 - 2023)	Eduardo G. de Campos
P14	Integration of Ultrashort-Chain Compounds into the Biomonitoring of Per- and Polyfluorinated Substances in Human Plasma and Serum	Shun-Hsin Liang
P15	Assessment of Δ9-THC and Δ9-THCCOOH Bias, Precision, and Ionization Suppression/Enhancement between Solid Tissue Homogenate and Supernatant by LC-MS/MS	Michael Fagiola

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P16	Clinical and Toxicological Presentation of an Accidental “Tranq Dope” Fatality	Elizabeth A. Taylor
P17	Quantification of Daridorexant, Lemborexant, and Suvorexant in Whole Blood using Liquid Chromatography Tandem Mass Spectrometry (LC-MS/MS)	Munchelou Gomonit
P18	Utility of a ketone body color test in identifying acetone-positive cases in vitreous humor compared to HS-GC/FID in postmortem toxicology	Crystal Arndt
P19	Stability of Fentanyl in Fortified and Authentic Postmortem Blood	Lisa M. Bianco
P20	A Toxicological Investigation amongst the Homeless Population in Jefferson County, AL	Lisa M. Bianco
P21	Semi-quantitative multiplex screening of 29 drugs from a single blood sample on the Evidence MultiSTAT biochip analyser	Roberta Grieger-Nimmo
P22	Development of an ELISA for the in vitro determination of Ethyl Glucuronide in oral fluid	Jessica Sprague
P23	Rapid and easy method for the determination of cocaine and six metabolites in post-mortem hair	Ana Pego
P24	Comprehensive LC-MS/MS Method for the Analysis of 94 Drugs of Abuse in Urine	Layla Cosovic
P25	LC-MS/MS Analysis of 14 Antipsychotic Drugs in Serum Utilizing Weak Cation Exchange SPE in a Miniaturized Microelution Format	Shahana Huq
P26	Validation of a Qualitative Immunoassay for 21 Drugs of Abuse in Oral Fluid on a Single Biochip Array	Alison Lightfoot
P27	Propoxyphene - Gone but not Forgotten	Wilsa J. Raymonvil
P28	Analysis of Δ-8-THC, Δ-9-THC, and Their Metabolites in Whole Blood by LC-MS/MS	Haley Berkland
P29	Multi-Class Drug of Abuse Rapid Screening in Urine, Plasma, and Oral Fluids Utilizing Paper Spray Mass Spectrometry	Jingshu Guo
P30	Old Dogs and New Tricks: A Fatal Case Involving Traditional Designer Drugs and an Emerging NPS Benzodiazepine	Paul Simmons
P31	Aptamer-based on-site colorimetric tests for opioids	Yi Xiao
P32	Quantitative Analysis of Δ8- and Δ9-Tetrahydrocannabinol Metabolites and Isomers: A Rapid Assay in Urine by LC-MS/MS	Marco Ballotari

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P33	Analysis of Phosphatidylethanol (PEth) in postmortem decomposition blood samples with corresponding vitreous humor ethanol concentrations	Carrol Nanco
P34	Withdrawn	
P35	An overview of mitragynine and fentanyl positive postmortem casework in Dallas County	Erin Jeffrey
P36	American Molecular 10 mL Tubes, A Candidate for Human Performance Testing	Katelyn Harvey
P37	Foam Cones and Opioids – Part Deux	Erin C. Strickland
P38	Unraveling Drug Facilitated Sexual Assaults: Insights from Harris County, Texas, 2023	Jeanna Mapeli
P39	Fentanyl concentrations for peripheral blood and brain samples in non-medical post-mortem cases	Kacey Cliburn
P40	Detection of Forensically Relevant Nitazenes Using Liquid Chromatography Tandem Mass Spectrometry (LC-MS/MS)	Sara Kuberski
P41	Qualitative Detection of Novel Urine Synthetic Cannabinoids and Positivity Rates	Ihuoma Igwilo
P42	Postmortem Distribution of Fentanyl and its Metabolites	Austin Ciesielski
P43	Novel LC-MS/MS Screen of Designer Stimulants in Urine Method	Nicole Nazario Bayon
P44	Drugs of abuse detected in oral fluid specimens of drivers in the state of Victoria (Australia) from 2008-2022	Elizabeth Jenkins
P45	Triggered multiple reaction monitoring LC-MS/MS method for screening of 90 drugs of abuse (including NPS) in hair	Siti Aisyah Jamil
P46	The Development of a Hair Inventory Tool and its Importance to Drug Testing in Hair	Lawrance Mullen
P47	Non-selective Sample Preparation Approaches Using Extrahera® Automation for Broad-spectrum DoA Whole Blood Analysis	Kyle Dukes
P48	Stimulants and Hallucinogens – Expanding efficiency and scope using Liquid Chromatography-tandem Mass Spectrometry	Alison Goetz
P49	Identification of Bongkrekic Acid in Postmortem Samples from a Foodborne Poisoning Incident in Taiwan	TE-I WENG

P50	Consolidating LC-MS/MS Method Conditions for the Analysis of Alcohol Metabolites, Barbiturates, and Drugs of Abuse	Paul Connolly
P51	Expanded Cannabinoid Analysis in 1300 Urine Specimens	Amy Patton
P52	Development and Validation of a Simple LC-MS/MS Drug Screen for 65 Compounds in Blood and Urine	Jessica Ayala
P53	Prevalence and Quantitative Analysis of 2F-2-oxo-PCE in Toxicology Specimens Collected in the United States	Alyssa Reyes
P54	The Rise of Bromazolam in Postmortem Cases from Travis County, Texas and Surrounding Areas	Christina Smith
P55	β-Glucuronidase Enzyme Kinetic Analysis for Applications in Urine Samples	Claire Collins
P56	Chemical and aerosol yield characterization of e-liquids: A 12-month study in parallel to participant use	Laerissa Reveil
P57	Benzodiazepine Trends in Maryland Postmortem Cases: December 2023 – March 2024	Zhenqian Zhu
P58	Novel Psychoactive Substances Most Commonly Identified in Clinical Urine Specimens from November 2023 to April 2024	Jillian Neifeld
P59	Carfentanil and Other Fentanyl Analog Prevalence in Clinical Urine Specimens Analyzed Using a High-Throughput Novel Psychoactive Substances Testing Panel	Theresa Meli
P60	4-ANPP: The Caution Flag for Illicit Fentanyl	Nicholas Laraia
P61	Breaking Bad: Methamphetamine's Role in Homicides	Rocio Potoukian
P62	The Analysis of Drugs of Abuse (DoA) and Novel Psychoactive Substances (NPS) in Oral Fluids by LC-MS/MS	Alexander Sherman
P63	Meeting National Safety Council Recommendations: Accurate Rapid Tests and Laboratory Confirmation Procedures for Fentanyl and Prevalent Opioids in Oral Fluid – Part 1	Christine Moore
P64	Meeting National Safety Council Recommendations: Accurate Rapid Tests and Laboratory Confirmation Procedures for Fentanyl and Prevalent Opioids in Oral Fluid – Part 2	Cindy Coulter
P65	Suspected occupational exposure to fentanyl & derivatives: responses from a pilot survey of emergency physicians	Oliver Grundmann

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POSTER SESSION #2
THURSDAY, OCTOBER 31, 12:00-2:00 PM
MODERATORS: ERIN JEFFREY AND KRISTIN KAHL

#	TITLE	SPEAKER
P66	Xylazine and 4-Hydroxy Xylazine Addition to Definitive Drug Testing Panel for Human Urine	Thomas G. Rosano
P67	Analysis of 15 cannabinoids in postmortem fluids and tissues using UPLC/MS/MS	Sunday Hickerson
P68	Profiling Heavy Metals in Kratom Powder Purchased in Southeastern Pennsylvania and Southern New Jersey	Xavia Pough
P69	Flash Enzymatic Hydrolysis Protocols for Different Drug Class Using B-One®	Elías Villalobos
P70	Direct-to-Definitive Drug Testing in Oral Fluid: Development of a Convenient Passive-drool Collection Technique and Evaluation of Analyte Stability	Thomas G. Rosano
P71	DUI Snapshot: Uncovering Drug Prevalence Trends in Washington, D.C.	Kimberley Heine
P72	The Rise of Nitazenes in Alabama	Amanda Cetnarowski
P73	A rapid and sensitive UHPLC System-MS/MS method for the analysis of traditional and novel benzodiazepines in Urine	Emily Lee
P74	Drug Surveillance in Oral Fluid Samples Collected from New York City Nightclubs	Brianna Stang
P75	Professional Mentoring Program Progress: 2020-2023	Kaitlyn Palmquist-Orlando
P76	Microsomes and Microchips - Proof of Concept of an Easy-to-use Workflow for Rapid Elucidation of Metabolites for MS-based Screening	Jürgen Kempf
P77	Rapid Seized Drug Analysis by RADIAN ASAP MS and Confirmation by High Resolution Mass Spectrometry	Emily Lee
P78	UHPLC-TOF-MSE Analysis of a Cohort of Patient Urine Samples from Drug Rehabilitation Centre's and an Emergency Department	Jonathan Danaceau
P79	Unveiling Metabolite Features of Synthetic Cannabinoids Suitable for Urine Screening - T-ReXing UHPLC-timsTOF-MS Data of pHLM Assays	Jürgen Kempf
P80	Evaluating Drug Positivity at BACs of >0.10 g/100 mL Across Five States	Grace Cieri

P81	Investigation of the effect of three solvents on the quantitative results for 7-Aminoclo-nazepam.	Leanne Hazard
P82	Intelligent Reflex Automatic Worklist Intervention for Toxicological Drug Screening by High-Resolution LC/Q-TOF	Cate Simmermaker
P83	Non-Nicotine E-Cigarette Products Branded as Personal Diffusers	Abby M Veeser
P84	Ultra-fast Online SPE LC/MS/MS for the Simultaneous Analysis of Drugs in Human Plasma Using the Multiplexed 4-channel System	Eishi Imoto
P85	Acetyl Fentanyl Detection in Umbilical Cord Tissue: Prevalence of Prenatal Exposure and Co-Exposure with Other Substances in a High-Risk Population	Liaqat Abbas
P86	Validation of ELISA Kits for Oral Fluid Screening	Rebeca Mella
P87	Comparative Analysis of Oral Fluid Drug Testing Methods: Sensitivity and Specificity Insights for 6-Acetylmorphine and Fentanyl	Tina German
P88	Mass spectrometric screening for drugs in urine in the clinical toxicology laboratory	Lixia Chen
P89	Bromazolam: Tracking Designer Benzodiazepines in Oral Fluid	Corey Widman
P90	Gabapentinoids Increasing in Montana	Elizabeth Holom-DeYoung
P91	Detection of Pharmaceutical and Illicit Drugs in Municipal Wastewater Using Gas Chromatography-Mass Spectrometry	Will Harris
P92	Development and Validation of Liquid Chromatography Quadrupole/Time of Flight (LC/QTOF) Drug Screening Method for over 375 Drugs in Blood using Size Exclusion Chromatography (SEC) at ANSI/ASB Recommended Screening Thresholds	Celia Modell
P93	Automated Workflows for Solid Phase Extraction of Fentanyl Analogs, Xylazine and Nitazenes in Urine and Oral Fluid	John Laycock
P94	The Analysis of Tianeptine in Whole Blood and Urine by Reverse-Phase SPE and LC-MS/MS	Ritesh Pandya
P95	Detection and Quantification of Carbon Monoxide in Postmortem Liver Tissues via Headspace GC-MS as an Alternative to Blood	Aracelis Velez
P96	Analysis of Synthetic Cathinones From Blood and Urine Using Clean Screen® Xcel I on LC-MS/MS	Ritesh Pandya
P97	Determination of the Aerosolization Efficiency of Nicotine and Ethanol in a Eutectic Mixture in E-liquids	Jessemia Meekins

P98	If You Can't Be With the Ethanol You Love, Love the Methanol You're With: A Look at Methanol Poisoning Cases in Miami-Dade County	Jennifer Gonyea
P99	Carfentanil Goes West	Vanessa Meneses
P100	The Heat Is On - Pushing Detection Limits of LC-QTOF-MS Screening with a VIP-HESI Source	Jürgen Kempf
P101	Cannabinoid Cross-Reactivity Using Four Oral Fluid Instant Test Devices	Jake Johnsick
P102	An Analysis of Drug Detections in Carfentanil Cases Across the Country from 2020-2024	Stuart Kurtz
P103	Bromazolam and Fentanyl in Postmortem Blood Samples	Melanie Belinsky
P104	GC-MS Analysis of Tetrahydrozoline in Alcoholic Beverage Residue Related to Drug-Facilitated Sexual Assault	Alyssa Hampton
P105	An Automated Dispersive Pipette Extraction for Sensitive Analysis of Alcohol Metabolites in Common Forensic Matrices	Madison Webb
P106	Development and Validation of a LC-MS/MS Method for the Quantitation of Synthetic Cathinones	Hunter Fleming
P107	In vitro evaluation of the toxicity of synthetic psychoactive cathinones	Sydnee Dressel
P108	Distribution of Protonitazene, Bromazolam, and their Metabolites in a Fatal Overdose: A Case Study	Danai T. Taruvinga
P109	Impaired Driving Surveillance in Arkansas: A Collaboration between the Arkansas State Crime Laboratory and Glen F. Baker Public Health Laboratory.	Kenton Leigh
P110	Evaluating the performance of commercial nitazene immunoassay test strips for drug checking applications	Liam M. De Vrieze
P111	Identification of 15 Urinary Biomarkers for Cannabis Exposure Across Various Cannabis Users	Marquise Xavier
P112	The Comparison of Whole Blood and Vitreous Fluid Drug Findings in Fifty Postmortem Cases.	Laureen Marinetti
P113	A Quantitative Separation Workflow for Δ8, Δ9 & Δ10-THC Analytes and their Respective Metabolites Extracted from Whole Blood Using Enhanced Matrix Removal and LC/MS/MS.	Peter Stone
P114	A Unified Liquid Chromatography-Tandem Mass Spectrometry Method for the Quantification of Seven Ethanol Biomarkers in Blood and Oral Fluid	Alaina Holt

P115	Withdrawn	
P116	Characterization of oral nicotine pouches: An evaluation of biorelevant in vitro release, pH, and nicotine content	Laerissa Reveil
P117	Validation of an analytical method for quantitation of Xylazine in human umbilical cord tissue	Andre Sukta
P118	Application of Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS) for the Identification and Determination of Narcotic Substances in Human Nail Samples from Jordanian Subjects	Abdelqader Asad
P119	Detecting Novel Psychoactive Substances Through the Use of a Surveillance Library: a 2023 Review	Chelsey Deisher
P120	Evaluation of Methamphetamine Positivity in the DUID Population (2018 - 2023)	Victoria Hill
P121	Z-Drug Concentrations in Postmortem Investigations, 2021-2023	Stephanie Marco
P122	Determining the detection of 215 Fentanyl Analogs and Synthetic Opioids using Four Commercial Immunoassays	Carl Wolf
P123	Design of Experiment Studies to Evaluate Extraction Parameters for the Detection of Chronic and Single Dose Drug Exposure in Forensic Hair Analysis	Kaylyn Keith
P124	Characterization of Xylazine’s Rise within San Francisco	Tyler Devincenzi
P125	The Evaluation of “Hemp-derived” Cannabis Product Mislabeling Using LC-MS/MS	Ashleigh Outhous

Successful application of a prediction model for the direct alcohol biomarker phosphatidylethanol 16:0/18:1 (PEth) in a judicial context

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Introduction: Longitudinal (i.e. over time) monitoring of ethanol abstinence is relevant in various contexts, such as driver's license regranting, custody and familial court cases, workplace testing, etcetera. Phosphatidylethanol 16:0/18:1 ('PEth') is a valuable direct biomarker for ethanol intake that can be measured in blood. For this purpose, blood can be conventionally sampled via a venous blood draw or, alternatively, it can be collected following a fingerprick, using volumetric absorptive microsampling (VAMS), in which a polymeric tip wicks up 10 µl of blood, which is subsequently dried, yielding dried blood microsamples. Based on a large-scale population study, we developed a prediction model to confirm ethanol abstinence or minor ethanol intake, even when consecutive positive PEth values are available (Van Uytfanghe et al., Addiction, 2022, https://onlinelibrary.wiley.com/doi/10.1111/add.15811). In addition, we validated the use of 20 ng/mL as a suitable cut-off below which compatibility with ethanol abstinence or minor ethanol intake can be concluded (Van Uytfanghe & Stove, Clinical Chemistry, 2023, https://doi.org/10.1093/clinchem/hvad096).

Objectives: Since 2019, our Laboratory at Ghent University, Belgium, has routinely performed assessments of PEth, primarily in the framework of driver's license regranting. PEth is determined in dried blood microsamples via an ISO 17025-accredited fully validated liquid chromatography - tandem mass spectrometric procedure. Four years post-implementation, we evaluated the use of PEth as a biomarker for longitudinal monitoring of ethanol abstinence and performed an independent validation and update of the prediction model that allows to conclude abstinence.

Methods: Data from April 2019 – February 2024 were stratified in 2 groups: subjects sampled via fingerprick under our direct supervision (group 1), versus subjects from whom a sample arrived via a clinical laboratory (group 2). To be considered for inclusion for the evaluation of longitudinal monitoring, subjects needed to have been sampled 3 times or more, and the time between consecutive samplings should not have exceeded 40 days. Results were evaluated for their compatibility with abstinence or minor alcohol intake according to either the application of the prediction model (in case of PEth values >20 ng/mL) or the 20 ng/mL cut-off, following the hypothesis that all subjects should be or should become abstinent from the first sampling moment on. In addition, datasets that complied with the prediction model were used to further update the model.

Results: From the 1848 unique subjects (group 1: 527, group 2: 1321) that had been sampled, 163 (group 1: 112, group 2: 51) met the inclusion criteria for the evaluation of longitudinal monitoring. In group 1, 72.3% of the subjects had results compatible with abstinence, 6.3% were able to remediate by the third sampling and 21.4% did not become abstinent. More than 50% of all subjects had more than 3 samplings, with ~13% (15/112) eventually returning to social drinking. In group 2, 51.0% of subjects had results compatible with abstinence, 13.7% were able to remediate by the third sampling and 35.3% did not become abstinent. More than 50% of the subjects of group 2 had more than 3 samplings, with ~18% (9/51) eventually returning to social drinking.

For all subjects, we observed that in 95% of the cases where the first two samplings were compatible with abstinence, the third sampling was compatible with abstinence as well. If the first two samplings were not compatible with abstinence, for group 1, 100% maintained the same status, while for group 2, 31% remediated.

Results for subjects that complied with the prediction model (32 in total) were used to evaluate and update the prediction model. It was confirmed that the prediction interval did not change by adding additional data and that for none of the subjects a steeper decrease in PEth was observed compared to the original dataset. The update allowed us to extend the time period in which the model is applicable, from 30 to 35 days.

Discussion: After 4 years of experience of using PEth as a longitudinal biomarker for ethanol abstinence, the evaluation is unequivocally positive. The majority of the subjects that were sampled under direct supervision of experts succeeded to be and remain abstinent, in contrast to those subjects that received their results via an electronic platform (the clinical laboratories). This highlights the added-value and/or motivation that can be found with directly supervised sampling.

S2

Addressing QC Tracking and Measurement Uncertainty With Simple Excel Spreadsheets, Without Automation or Macros

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Introduction: Accreditation according to ISO17025 requires tracking and evaluating QC results and calculation of measurement uncertainty for every quantitated analyte. In many laboratories newly seeking ISO accreditation, the goal is to meet the requirements with a minimal amount of added work and with minimal disruption to laboratory workflows. Although there are several solutions for both QC tracking and measurement uncertainty, commercial solutions might be less suitable for the smaller laboratory as they require integration with instrument software or coordination with IT departments to adopt, include manual calculation steps, are not specific enough to toxicology workflows, and/or come with licensing fees. Microsoft Excel is a relatively ubiquitous and accessible calculation tool which represents a more feasible option; however, establishing a robust and effective system often requires advanced knowledge of formulae and/or macros which may be beyond the level of laboratory staff. Despite training, workshops, and collaboration among colleagues, it is often challenging to implement the strategies and calculations adopted by other laboratories.

Objectives: To develop spreadsheets and accompanying workflow to meet ISO17025 requirements for QC tracking and measurement uncertainty calculations that are a) easy and efficient to use; and b) do not require expert Excel knowledge such as macros or automation.

Methods: There are many requirements for an ideal QC tracking spreadsheet, including ease of data entry, allowing blank/missing data, plotting without manual steps, and calculation of CV and bias. The ideal spreadsheet must be able to register and plot data from multiple QC levels and provide meaningful plots, both with few and many data points.

Similarly, the measurement uncertainty spreadsheet must adhere to forensic toxicology best practices (such as the draft ASB 056 standard), include all calculations without manual intervention, and reduce duplicate entries to a minimum when working with a large number of drugs and matrices.

Results: For QC tracking, a spreadsheet was established per method, with a tab for each drug. Results for multiple QC levels are entered together to simplify and streamline data entry, and a single plot is created using different colors for different levels. This allows for the evaluation of both global and QC level-specific trends. For each QC level, the n, CV and bias are calculated without user action.

The spreadsheet for measurement uncertainty is in agreement with ASB 056 and separates contributors into laboratory-wide, method-specific, and analyte-specific, providing all the necessary calculations by simple dropdown menus. The accompanying workflow describes how the laboratory calculated all contributions, including pipettes, balances, glassware and dilutions.

Discussion: Both spreadsheets were evaluated and found acceptable in a recent ISO17025 inspection, and the measurement uncertainty spreadsheet was also capable of replicating the results of the appendices in draft ASB 056 within 0.1% (acceptance criteria allowed for rounding differences between Excel [which carries all digits throughout calculations] and the ASB document). Neither spreadsheet uses macros or requires automation, to allow for easy integration and standalone operation—thereby ensuring that they will work without expert knowledge. All calculations are done by the spreadsheets without manual steps, and the calculation cells are locked to avoid accidental changes.

As part of this presentation, both spreadsheet templates and model instructions will be made available to attendees; and the authors are willing to assist in laboratory implementation.

S3

International Cooperation Supporting the Development of Forensic Science

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Introduction: The International Criminal Investigative Training Assistance Program (ICITAP) is a law enforcement development organization within the U.S. Department of Justice and works with foreign police and forensic laboratories to improve global security and counter transnational crime such as drug trafficking, terrorism, and corruption. Forensic science is a key component of ICITAP efforts. It promotes fair and effective criminal justice systems as well as improved public safety, each of which are essential foundational pieces to broader economic development. ICITAP cooperates with partner laboratories around the globe to achieve international accreditation and other process improvements and plans to make efforts to support the Department of State's Global Coalition on Synthetic Drugs.

Objectives: The objectives of this presentation are to demonstrate the importance of forensic science to the international development community and review ICITAP's approach to facilitating foundational change of the practice of forensic science in foreign laboratories. The presentation also aims to outline opportunities for future engagement between SOFT members and foreign counterparts.

Discussion: An overarching goal of ICITAP's forensic assistance programs is international accreditation and adoption of recognized best practices. To date, ICITAP has supported the accreditation of Latin American forensic laboratories in over 15 countries, including over 20 Mexican states. Accredited forensic disciplines include DNA, chemistry/toxicology, fingerprints, firearms, questioned documents, digital evidence, crime scene, anthropology, and medicolegal death investigations.

Accreditation requires not only a significant investment in money and time, but the desire for organizational change to include modifications in work processes and laboratory culture. The challenge is even greater when a laboratory seeks to become the first accredited forensic laboratory in their country or region. The full support of the institution's senior leaders is needed to align the laboratory culture with a change of this nature as well as provide solid management skills including strategic planning, budgeting, and administration of a rigorous quality assurance program. Additionally, staff scientists must embrace a system that requires greater transparency and engaging in quality management practices daily.

As a complement to ICITAP's quality-related assistance, the presentation will review ICITAP's efforts to address the timeliness of results needed to be an effective laboratory. While reliable evidence is needed for criminal trials, timeliness is needed to aid public health as well as criminal investigations that lead to prosecution. Unfortunately, quality and timeliness are often in tension. The presentation will review ICITAP's support of Lean Six Sigma training for Costa Rica complementing their push for accreditation. As a result of this assistance, Costa Rica was able to reduce their backlog of cases in the laboratory more than 90 days by 95 percent.

Lastly, the presentation will highlight ICITAP's specific efforts related to collaborative assistance supporting death investigations in Latin America to include a series of observerships for Latin American toxicologists and pathologists in Arkansas and the District of Colombia.

SOFT members can potentially contribute to ICITAP's work through hosting foreign scientists in their laboratory for observerships or serving as an advisor during short-term assignments in another country. Short-term assignments may focus on a number of topics to include, but not limited to, mentoring on method development, validation, and other quality-related issues.

S4

Rebranding the Postmortem Toxicology Laboratory: Creating New Partnerships within Public Health

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Introduction: During and after the height of the COVID-19 pandemic, public health laboratories faced challenges. Already understaffed divisions held together by underpaid employees were stretched thin as the promise of work from home employment enticed workers to leave government institutions in large numbers. In North Carolina, the state-wide medical examiner's system currently exists under the Department Health and Human Services (DHHS), Division of Public Health (DPH), which naturally creates challenging layers of bureaucracy. Even with support units such as human resources, information technology, and purchasing, adequate and appropriate staffing has been a challenge. During the period from 2022-2024 NC OCME has undergone a series of strategic shifts to clearly define our mission, increase employee retention, increase available resources and improve overall product quality.

Objective: In this presentation, attendees will learn how our laboratory rebranded itself to revitalize our mission and purpose by embracing a more clinical identity, growing partnerships, and using creative thinking to patch together existing resources to create a brighter future.

Method/Results: Information was gathered from an agency satisfaction survey which led us to better understand pre-existing misconceptions, even within our own division. Increasing transparency and building communication bridges was necessary because of the expansive state-wide services the laboratory provides and it's many and diverse stakeholders. The increase in public relations and partnerships, including regular participation in various state Task Force meetings (Child Fatality, Impaired Driving) and increased data sharing between state agencies, facilitated trust and appreciation of our work product. One specific example is our collaboration with the state Injury and Violence Prevention Branch. By providing deidentified postmortem data, their staff of epidemiologists produce monthly reports for overdose surveillance efforts. This customer-service based approach has benefited our team in many ways, including an increase in funding specifically for the laboratory which has been used to address technology, instrumentation, and resource needs.

As the period of our previous accreditation was scheduled to expire, NC OCME made the decision to opt for College of American Pathologist (CAP-LAP) accreditation. This being the result of the careful consideration of our role and future goals. As a postmortem lab within the DHHS, our analysts rarely testify in court, and our data is often used in a more clinical capacity. This CAP accreditation process resulted in a thorough inventory of lab resources, put an emphasis on safety, and increased regular competency checks of all employees, leading to an overall improvement in lab quality.

To respond to under-staffing, the restructuring of our scientific team has included embracing temporary and time-limited positions, hiring a program coordinator, and the creation of an entirely new senior position of Forensic Toxicologist. This has helped to remediate immediate staffing shortfalls and has aided in future efforts to create multiple career paths at the senior and supervisory level.

Strategic shifts have been made to our initial screening strategy to allow full-scan high resolution mass spectrometry. The hope is that this will allow a multi-tier screening process, where expanded priority targeting will increase the quality of the toxicology results reported to pathologists and families, and expanded retrospective data mining can help with real-time surveillance efforts to track new drug emergence in North Carolina for epidemiological use.

Discussion/Conclusions: We continue to strive to be valued within our state as a leader in laboratory science, and to avoid being confused with the state crime lab or the state laboratory of public health. By sharing the management lessons learned along the way, the hope is that other government funded laboratories can hear what did work, what (really) didn't work, and what we are striving to achieve in the future.

Nitazenes of the past, present, and future: Insights from *in vitro* μ-opioid receptor assays and *in vivo* behavioral studies in mice

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Introduction: New synthetic opioids (NSOs) continue to contribute to drug-related fatalities worldwide. Since 2019, various NSOs with a 2-benzylbenzimidazole scaffold ('nitazenes') have been detected in the recreational drug market. While new to the recreational drug market, different nitazenes (e.g., isotonitazene) were originally studied in the 1950s-60s for their potential as analgesics. Others, such as *N*-pyrrolidino protonitazene, have never been evaluated before, and are truly 'new' synthetic opioids. As the group of nitazenes continues to expand, these substances pose an increasingly significant threat to public health worldwide. In this work, we synthesized and characterized 15 nitazenes differing in 4 positions of the 2-benzylbenzimidazole structure (R_1-R_4). These 'prophetic' analogues were selected from old literature or newly designed based on their predicted biological activity and/or relative ease of synthesis.

Objectives: The results aim to expand the existing knowledge about 2-benzylbenzimidazole structure-activity relationships (SAR), while also helping stakeholders (e.g., forensic toxicologists, clinicians, policymakers) in their risk assessment and preparedness for the potential next generation of nitazenes.

Methods: *In vitro* characterization focused on the μ-opioid receptor (MOR), the primary molecular target for clinically applied and abused opioids. MOR affinity was determined via competition radioligand ([^{3}H]DAMGO) binding assays in rat brain tissue. MOR activation (potency and efficacy) was studied by means of a cell-based β-arrestin 2 recruitment assay. To complement *in vitro* findings, *in vivo* experiments were performed to investigate opioid-like effects in male C57BL/6J mice for seven analogues of the highly potent etonitazene.

Results: Binding assays revealed that all nitazenes bind to MOR with nanomolar affinities (K_i=8-431 nM). In the functional assay, potencies ranged from 0.588 nM (etonitazene) to 1266 nM (ethylene nitazene). Methionitazene (EC_{50}=5.28 nM) and α'-methyl etonitazene (EC_{50}=1.00 nM) exceeded the potency of fentanyl (EC_{50}=17.0 nM). Most newly studied nitazenes were less active than their corresponding comparator 2-benzylbenzimidazole; notable exceptions were iso-butonitazene (EC_{50}=10.3 nM) and sec-butonitazene (EC_{50}=7.62 nM), the potencies of which exceeded that of butonitazene (EC_{50}=34.2 nM). Furthermore, methylnitazene (EC_{50}=70.2 nM) and propylnitazene (EC_{50}=9.50 nM) were more potent than nitazene (EC_{50}=312 nM). Efficacies ranged from 187-254% (compared to hydromorphone) in the employed assay. *In vivo*, dose-dependent effects were observed for antinociception, locomotor activity, and body temperature changes in mice. The antinociceptive potency of etonitazene was 0.022 mg/kg. The most and least potent analogues were α'-methyl etonitazene (ED_{50}=0.060 mg/kg) and ethyleneoxynitazene (ED_{50}=11 mg/kg), respectively. Bell-shaped curves were obtained for locomotor activity. The maximum distance traveled by the animals was largely comparable between the treatment groups (~320-410 m), and all analogues induced a comparable maximum decrease in body temperature compared to baseline (2.0-2.6°C).

Conclusion/Discussion: As nitazenes are increasingly present at the street level – as individual drugs, mixed with or mis-sold as e.g. heroin, or as ingredients of falsified medications – close monitoring of market developments and rapid characterization of emerging analogues is of critical importance. By expanding our knowledge of nitazene SARs, this study aims to inform relevant stakeholders regarding nitazenes that have been actively contributing or might be anticipated to contribute to the nitazene as well as the larger opioid crisis.

S6

In vitro metabolism of Δ8-tetrahydrocannabinol (Δ8-THC) and Δ8-THC-O acetate (Δ8-THCO)

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Introduction: Isomers of Δ9-tetrahydrocannabinol (Δ9-THC), the principal psychoactive substance in cannabis, have gained widespread attention recently. Among them, Δ8-tetrahydrocannabinol (Δ8-THC) has emerged as a popular recreational cannabinoid, while THC-O acetate (Δ8-THCO), a synthetic derivative of Δ8-THC has also raised significant scientific interest.

Objectives: This study aimed to investigate the enzymes involved in the metabolic pathways of Δ8-THC and Δ8-THCO. Understanding these pathways is crucial for assessing adverse drug reactions, drug-drug interactions, and potential toxicity.

Methods: The research investigated the metabolism of Δ8-THC and Δ8-THCO using pooled human liver microsomes (HLMs), pooled human plasma, recombinant cytochrome P450 (rCYP) enzymes, and human carboxylesterases (hCES). Stability and adsorption studies of Δ8-THCO were also conducted to ensure the validity of results. Liquid chromatography-quadrupole/time-of-flight-mass spectrometry (LC-Q/TOF-MS) and liquid chromatography-tandem mass spectrometry (LC-MS/MS) were used for analysis. Additionally, inhibition studies were performed to validate the specific enzymatic pathways involved.

Results: The high lipophilicity of Δ8-THCO led to reduced signal intensity due to surface adsorption on plastic microcentrifuge tubes. This was mitigated by conducting enzymatic incubations in borosilicate glass tubes. Pooled human liver microsomes exhibited similar metabolic profiles for both Δ8-THC and Δ8-THCO, indicating stepwise metabolism of Δ8-THCO to Δ8-THC and its corresponding hydroxylated species, including 11-hydroxy-Δ8-THC. While Δ8-THC primarily underwent hydroxylation via cytochrome P450 (CYP) enzymes (2C9, 2C19, 2D6, and 2J2), Δ8-THCO did not undergo CYP-mediated deacetylation, but showed substantial desaturation, forming novel desaturated compounds. Human carboxylesterases (hCES1b, hCES1c, and hCES2) actively catalyzed Δ8-THCO to produce Δ8-THC, highlighting their role in Δ8-THCO metabolism. Confirmation of enzyme activity was obtained using corresponding inhibitors.

Discussion: This comprehensive analysis not only enhances our understanding of the metabolic fate of Δ8-THC and Δ8-THCO, but also highlights the intricate interplay between cannabinoids and metabolic enzymes. Identification of previously unreported CYPs involved in Δ8-THC and Δ8-THCO metabolism adds valuable insights, although some specific metabolites were not identified in the HLM profiles. This knowledge is invaluable for studying minor cannabinoids, unraveling their transformations, understanding their physiological effects and potential risks, thereby guiding future policy decisions.

The Prevalence of Delta-8-THC in Orange County, CA

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Introduction: With the current legalization status of Cannabis in California, there has been an increase in Cannabis products available to consumers, including both delta-8-tetrahydrocannabinol (delta-8-THC) and delta-9-THC. This increase lead to the laboratory developing and validating a qualitative liquid chromatography tandem mass spectrometry (LCMSMS) method to differentiate the two isomers and their respective carboxy metabolites since the original validated quantitative method was unable to separate them. Without the ability to separate and report each isomer, it may be difficult to interpret cases as more information is published for the individual isomers.

Objectives: The objective was to review cases previously analyzed for delta-8-THC isomers and discuss the prevalence and relevance of having identified them in post-mortem (PM) and driving under the influence of drugs (DUID) cases.

Methods: All samples were screened presumptive positive for Cannabinoids by Immunoassay with Immunalysis® Direct ELISA Cannabinoids kit, then analyzed for delta-9-THC, 11-nor-9-carboxy-delta-9-tetrahydrocannabinol (delta-9-Carboxy-THC), 11-hydroxy-delta-9-tetrahydrocannabinol (Hydroxy-THC), cannabidiol, and cannabinol. An automated extraction is utilized using a Tecan Freedom EVO 200 and Biotage SLE+ 96 well plates with deuterated internal standards of all quantitative drugs. If interference in the delta-9-Carboxy-THC or delta-9-THC window is identified, the sample is injected on a separate qualitative LCMSMS method for both delta-8 and delta-9-THC along with their carboxy metabolites. Both analyses were performed on Waters Aquity UPLC using an HSS T3 1.8 µm, 2.1 x 50 mm column for the initial quantitation and a BEH C18 1.7 µm, 2.1 x 100 mm column for the isomer separation. The limit of detections were administratively set at 5 ng/mL for both delta-8 and delta-9-Carboxy-THC isomers, 2 ng/mL for the delta-8-THC and 1 ng/mL for the delta-9-THC. Both methods were validated following ANSI/ASB Standard 036, Standard Practices for Method Validation in Forensic Toxicology.

Results:

Table 1: Reported cases for each compound based from 2022-2024 (count (percentage of total cases)

	2022	2023	2024	Total
delta-9-Carboxy-THC	5157 (49%)	4754 (42%)	1095 (40%)	11006 (45%)
delta-9-THC	3354 (32%)	3710 (33%)	902 (33%)	7966 (32%)
Hydroxy-THC	1992 (39%)	2557 (22%)	666 (24%)	5215 (21%)
delta-8-Carboxy-THC	11 (<1%)	146 (2.3%)	37 (<3.1%)	194 (1.5%)
delta-8-THC	9 (<1%)	43 (<1%)	7 (<1%)	59 (<1%)

There were 253 total reported cases for both delta-8 isomer compounds with 2023 having the most complete compiled data. In 2023, there were five cases and currently one case in 2024 that were delta-8-THC only. For PM cases (2022-2024) there were three, 50 and eight cases, respectively, containing one or both delta-8 isomer compounds. For the same years, there were 17, 139 and 36 cases, respectively, for DUID cases.

Discussion: The validation for qualitative detection of the delta-8 isomers was implemented January 2023 after their appearance starting in 2018. Before implementation of the new method, some cases may have been reported as indeterminate if there was interfering peaks in the delta-9-THC and metabolite chromatography. There could be a lower prevalence of delta-8-THC with its validated LOD at 2 ng/mL versus 1 ng/mL for delta-9-THC. Delta-8-THC has similar symptomology on human performance and behavior to delta-9-THC, but is considered less potent; therefore, it may be relevant for interpretation for DUID cases. As a marijuana legal state, the prevalence is not as high as reported in other states where marijuana is either only legal for medicinal use or not at all. Delta-8-THC and its metabolite are commonly detected with ethanol and methamphetamine or often alone, similar to their delta-9-isomers. It would be important to revisit the results for 2024 to compare with 2023 since many of the drugs reported in DUID related cases often include Cannabinoids.

Non-fatal overdose bio-surveillance: A multi-site cross-sectional pilot study

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Introduction: Much is known about the risk factors and prevalence of polysubstance use in fatal overdoses. However, polysubstance use and other risk factors for experiencing a non-fatal overdose are less well-defined. Risk factors and information about substances associated with non-fatal overdoses are difficult to assess due to the prevalence of non-fatal overdose events that do not, or minimally, intersect with the medical system. Examining substances present in ED patients experiencing an overdose reveals a broader and more complex picture of the overdose epidemic than when solely focused on overdose deaths.

Objectives: This study aims to expand toxicological testing performed on non-fatal overdose specimens to inform community partners and the public about substance use trends and emerging drug trends within communities. Expanded testing may reveal novel psychoactive substances emerging in a community or subpopulation, enabling efficient community partner education and intervention.

Methods: In this multi-center cross-sectional pilot study, residual urine specimens from patients experiencing overdoses and seeking medical care at participating EDs were included from August 2022 through February 2024. This project was approved by the University of Wisconsin-Madison's IRB (ID #: 2022-0393). Urine specimens were re-analyzed for the presence of drugs of abuse and associated metabolites via a wide-scope data-independent acquisition (DIA) liquid chromatography-high resolution mass spectrometry (LC-HRMS) approach. Analytes were qualitatively identified by comparing retention time, exact mass, and fragmentation to an in-house spectral library containing over 800 relevant compounds. A limited data set was collected for each specimen including patient age, sex, race, ethnicity, clinical toxicology results, blood alcohol concentrations, what medications were administered to the patient before urine collection, and a summary of presenting symptoms and chief complaints. Data was also gathered on the manner of overdose and for the presence of several risk factors including if the patient was: unhoused, experienced chronic pain, had a HIV or Hepatitis B/C infection, was pregnant, had been incarcerated within the last 30 days, has a history of overdose requiring medical care, a history of substance misuse, or a mental health disorder. The outcome of the emergency department visit was also captured. Data was summarized as prevalence with 95% confidence intervals and significant associations were identified via cross-tabulation of two binary variables (so-called two-by-two tables). Odds ratios were also calculated to identify the directionality of any significant association.

Results: A total of 79 complete submissions were included in this work. Participant ages spanned from 1 to 70, with a mean age of 34. Both sexes were represented in this work, but most participants identified as White/Caucasian, followed by Black/African American, unknown, and American Indian or Alaskan Native. There were a few significant associations between demographic variables such as males being more likely to have detectable levels of any simulant-class drug or synthetic opioids whereas females were more associated with the presence of antidepressants. Some drugs or drug classes were significantly associated with risk factors such as narcotic analgesics or synthetic opioids in people with a history of overdose, any stimulant in people with a history of substance use disorder, antidepressants in people with a history of a mental health condition, and any stimulant and cannabinoids in samples indicating polypharmacy consumption behaviors. Considering polypharmacy, some drugs or drug classes were significantly associated, such as dissociative anesthetics with any stimulant or cocaine, narcotic analgesics with cocaine, and any stimulant with synthetic opioids. Cannabinoids were prevalent in people discharged from the ED and uncommon in people admitted to the hospital. Antidepressants were commonly identified in people admitted to the hospital.

Discussion: We identified several significant associations between patient demographics, risk factors, substances used, and ED encounter outcomes within the population of people who experience a non-fatal overdose. Directionality of significant associations indicates risk and protective factors associated with this understudied subpopulation and informs public health practitioners, medical providers, and the public about substance use trends and emerging drug trends within communities.

S9

Urinary Fentanyl Concentrations in Patients Dosed with Fentanyl from Transdermal Patches, Tablets, Lozenges, or Sublingual Spray

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Introduction: Fentanyl is a potent synthetic opioid often prescribed for pain management to be self-administered through various dosage forms, including transdermal patches, lozenges, sublingual sprays, and buccal tablets. In the wake of the opioid epidemic, education campaigns warning of the dangers associated with opioid use may impact patient adherence and compliance with medications such as fentanyl. Urine remains a reliable, non-invasive biological matrix routinely used to determine patient compliance of prescribed medications. This work reports results from a large number of patient urine samples from patients prescribed one of several forms of fentanyl for pain relief. Establishing both raw and normalized data ranges of urinary fentanyl concentration for commonly prescribed dosage forms of fentanyl will afford a reference data set for prescribers to determine if a patient is compliant; often without the need for patient reports or longitudinal data.

Objective: The purpose of this study was to characterize the urinary fentanyl concentrations of patients prescribed fentanyl to establish normal, reference ranges for fentanyl compliance.

Methods: Patient specimens were obtained from 6,285 routine urine screens and tested for fentanyl using liquid chromatography-tandem mass spectrometry (LC-MS/MS). Inclusion criteria consisted of patients who were prescribed fentanyl in a self-administered dosage form (transdermal patches, lozenges, buccal tablets, or sublingual sprays) and i) tested positive for fentanyl, ii) tested negative for illicit substances, and iii) included creatinine, age, BMI, sex, and fentanyl dose prescribed. Urinary fentanyl concentrations were normalized to creatinine and dose and further characterized by age, body mass index (BMI), and sex. Ages were grouped from <18, 18-64, and 64+ years old. BMI was grouped into <18.5, 18.5-24.9, 25-29.9, and 30+. Analysis of variance (ANOVA) with Tukey's Honest Significant Difference (HSD) was used to assess statistical differences of urinary fentanyl between age, BMI, sex, and dose form groups.

Results: Of the 6,285 specimens tested, 2,930 fit into the inclusion criteria, which consisted of 1,058 males and 1,872 females. Positive urine concentrations of fentanyl ranged from 3 ng/mL to 9,849 ng/mL. The age range of patients was between 19 and 98 years old with an average age of 59.70. The BMI of patients ranged from 11.90 (underweight) to 77.47 (obese), with an average BMI of 29.69. The most commonly prescribed dosage form was the transdermal patch, which accounted for 2,870 of the included specimens and was prescribed in doses ranging from 12 to 100 µg/hr. Lozenges, buccal tablets, and sublingual sprays accounted for 54, 4, and 2 of the specimens, respectively, with doses ranging from 100 to 1600 µg. Significant differences in urinary fentanyl were observed between transdermal patches, lozenges, and buccal tablets ($p < 0.05$). Significant differences in urinary fentanyl were also observed between age groups 18-64 and 64+ ($p < 0.05$). No significant differences were observed between males and females or between BMI groups.

Discussion: Characterizing the urinary fentanyl concentration of patients who were prescribed fentanyl, raw and normalized data ranges are presented. These ranges may help determine patient compliance with fentanyl from urine collected for non-invasive, routine testing.

S10

Adverse events and mitragynine and 7-hydroxymitragynine plasma pharmacokinetics after controlled mitragynine isolate to healthy human participants

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Introduction: KAPTURE is the first controlled study evaluating safety and tolerability of single (SD) and multiple (MD) mitragynine isolate doses. Mitragynine, a partial μ-opioid receptor agonist, does not recruit ß-arrestin2 of the respiratory depression pathway unlike traditional opioids, e.g. morphine. Mitragynine pharmacology includes antagonism at κ-opioid receptors and agonism at α-adrenergic, serotonergic, dopamine, and adenosine receptors.

Objectives: The purpose of this study is to provide the first safety and tolerability data and pharmacokinetics for mitragynine isolate to better understand mitragynine and 7-hydroxy-mitragynine (7-OH-MTG) effects.

Methods: The Advarra Institutional Review Board and Health Canada approved this study. A placebo-controlled, single (SD) and 15-consecutive daily doses (MD) of 11.15, 22.3, 44.6, or 89.2 mg oral mitragynine isolate was conducted. Forty-eight participants received active (12 participants received SD and MD at each dose) and 67 placebo. Adverse events (AE), comprehensive metabolic and hematology testing, and plasma sampling occurred during 31 in-person visits, with follow-up for 10 and 23 days after SD and MD, respectively. Respiratory rate, SpO_2, ECG, and vital signs were also assessed.

Results: There were no serious AE or deaths. There were 35 completers and 7 early terminations; 6 due to AE, including two participants with difficult blood collections, one with vertigo, and three with elevated AST and/or ALT enzymes. Participants reporting AE after mitragynine increased from 3 to 12 with increasing dose. At the highest 89.2 mg SD (n=12), 58.3% of participants reported somnolence, 41.7% dizziness and nausea, 33.3% feeling hot and feeling of relaxation, and 25% vomiting. After the highest MD (n=12), 66.7% of participants reported somnolence, 50% dizziness and nausea, 41.7% feeling hot, 33.3% feeling of relaxation and euphoric mood, 25% headache, ALT and/or AST, dry mouth, feeling abnormal and tremor, and 16.7% hypervigilance, pruritis, feeling drunk, decreased appetite, therapeutic response unexpected, tinnitus, hyperhidrosis and vomiting. There were no AE during follow-up. 29.9% and 41.0% placebo participants reported similar AE after SD and MD, respectively. Euphoria-related events occurred primarily at the highest MD. Dizziness and somnolence were the most commonly-reported terms in active and placebo groups. There was no evidence of meaningful abuse potential or a withdrawal syndrome indicative of physical dependence after SD, but there were more abuse potential-related AE following the highest MD. Effects typically were mild and appeared within 2h after mitragynine.

After 89.2 mg mitragynine isolate SD, median (range) mitragynine Cmax was 219 (105-445) ng/mL, Tmax 1.2 (0.5-3.0) h, AUC_{0-24} 806 (432-1937) h*ng/mL and T1/2 42.9 (17.8-79.8) h and the same parameters for 7-OH-MTG were 34.2 (16.2-60.9) ng/mL, 1.2 (0.5, 3.0) h, AUC_{0-24} 163 (88.5-307) h*ng/mL and 4.0 (2.7-24.0) h respectively. After 89.2 mg mitragynine isolate MD, median mitragynine Cmax was 336 (193-753) ng/mL, Tmax 1.0 (0.75, 1.7) h, AUC_{0-24} 1675 (848-2730) h*ng/mL and steady-state T1/2 was 44.5 (42.6-78.6) h and the same parameters for 7-OH-MTG were 42.6 (28.9-63.3) ng/mL, 1.7 (0.75, 3.0) h, 248 (112-354) h*ng/mL and 33.7 (4.3-135) h, respectively. After 89.2 mg MD, accumulation factors for Cmax and AUC0-24 were 1.7 and 1.7 for mitragynine and 1.2 and 1.3 for 7-OH-MTG. Steady-state was reached Day 7 MD for both compounds. 7-OH-MTG/mitragynine ratio was higher during SD than MD, and at the lowest rather than highest doses, with a median maximum ratio of 0.45 (0.18-0.56) 5h following 11.15 mg SD mitragynine. Following mitragynine isolate MD, the median maximum ratio was 0.30 (0.14-0.35) 4h after the 11.15 mg MD.

Discussion: These controlled human administration data demonstrate that mitragynine isolate was well-tolerated, with few abuse-potential related effects at oral doses up to 89.2 mg.

S11

Quantitative Analysis of Traditional Drugs and NPS in Blood Specimens Collected from Emergency Department Patients After Suspected Drug Overdose

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Introduction: Traditional drugs and novel psychoactive substances (NPS) are commonly encountered during forensic toxicology testing. This is especially common in postmortem toxicology. Less testing has focused on determining drug concentrations in suspected non-fatal drug overdoses. There is growing interest in monitoring the prevalence of drugs in these cases in order to understand the impacts of drugs on human health. Analyzing blood specimens collected from suspected overdose patients in a hospital setting provides useful information to help bridge this knowledge gap.

Objectives: This study applied broad-spectrum toxicological drug screening to patients experiencing suspected drug overdoses. Data collected included positivity data for a broad range of legacy and emerging drugs and quantitative data for specific drugs of interest (e.g., fentanyl, methamphetamine, cocaine, xylazine) An existing validated liquid chromatography quadrupole time-of-flight mass spectrometry (LC-QTOF-MS) method was used for screening, and a novel quantitative method using liquid chromatography tandem quadrupole mass spectrometry (LC-QQQ-MS was developed and validated for confirmation and quantitation. Correlation of toxicology results with patient clinical outcomes was evaluated to gain better insights regarding indicators of intoxication while longitudinal findings provided new understanding of drug trends, both geographically and temporally.

Methods: Blood specimens were collected from patients in emergency departments under IRB approval. They were stored refrigerated and shipped to our laboratory for testing. All specimens were subjected to a broad-spectrum drug screen using a Sciex X500R LC-QTOF-MS employing a database of more than 1,100 drug targets. Drug confirmation and quantification were performed using a Waters Acquity TQ-S Micro LC-QQQ-MS. The analytes included in this method were fentanyl, methamphetamine, xylazine, amphetamine, benzoylecgonine (BZE), norfentanyl, cocaine, and naloxone, using corresponding deuterated internal standards. The method was validated in accordance with the ASB standard for method validation. Calibration ranged from 1 to 100 ng/mL. Solid phase extraction was used for sample preparation. Chromatographic separation was achieved using an Agilent InfinityLab Poroshell C-18 120 (2.7 µm, 3.0x100 mm) analytical column and gradient elution of mobile phases A) 5 mM ammonium formate in Water, pH 3 and B) 0.1% formic acid in acetonitrile. The flow rate was 0.35 mL/min and injection volume was 5 µL.

Results: To date, 573 specimens have been analyzed. Cases originated from 17 US states. Fentanyl was the most observed drug, accounting for 81% positivity (n=462). Fentanyl blood concentrations ranged from 1-140ng/mL with a mean (standard deviation) of 8.7±12.7ng/mL. Norfentanyl accounted for 73% positivity (n=417) with blood concentrations ranging from 1-200ng/mL and a mean of 7.6±17.5ng/mL. BZE was the next most frequently observed analyte (54%, n=310, 1.1-1000+ng/mL, 295±290ng/mL) followed by naloxone (41%, n=235, 1-510ng/mL, 14±42ng/mL), methamphetamine (37%, n=216, 1-960ng/mL, 181±227ng/mL), amphetamine (35%, n=199, 1-280ng/mL, 33±46ng/mL), cocaine (34%, n=196, 1-320ng/mL, 28±57ng/mL), and xylazine (22%, n=127, 1-150ng/mL, 11±19ng/mL). All cases containing xylazine also contained fentanyl, accounting for 27% of positive fentanyl cases. Xylazine was more commonly observed in the eastern and midwestern regions. Methamphetamine was more commonly observed in western states while cocaine/BZE was more commonly observed in eastern states. Interestingly, only 45% of fentanyl cases contained detectable naloxone despite nearly all patients receiving naloxone administration in one form or another. A variety of NPS were qualitatively and quantitatively observed, including benzodiazepines, opioids, and synthetic cannabinoids.

Discussion: Our results from clinical toxicology testing after non-fatal overdose show high positivity for fentanyl and high co-occurrence with other drugs. Drug concentrations observed overlapped substantially with those seen in fatal cases. These analytical findings help illustrate what drugs are present in different geographic regions, popular drug combinations, and the challenges of interpreting postmortem and clinical drug concentrations in isolation.

Drugs of abuse influence inflammation profiles in deceased and living individuals

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Introduction: Cytokines are signaling molecules essential to immune system function. They are expressed under various endogenous and exogenous stimuli and are widely interconnected in how they modify immune system function. There is a wealth of research demonstrating that many therapeutic and illicit substances have immunomodulating effects that lead to altered cytokine concentrations both *in vitro* and *in vivo.* This led us to explore if drugs of abuse alter cytokine concentrations.

Objectives: Our objective was to evaluate if cocaine, amphetamines, fentanyl, or fentanyl plus xylazine alter inflammatory cytokine profiles in humans.

Methods: We evaluated three groups of samples. In group one we measured cytokines in the vitreous humor (VH) of decedents who tested positive for either cocaine, amphetamines, or fentanyl at the time of death. We tested 24 postmortem vitreous samples, with eight samples confirmed for each drug category. A negative control was comprised of 29 VH samples from individuals who succumbed to non-pathological causes. We measured the concentrations of interleukin 1 beta (IL-1β), interleukin 2 (IL-2), interleukin-6 (IL-6), interleukin (IL-15), Monocyte Chemoattractant Protein 1 (MCP-1), tumor necrosis factor alpha (TNF-α), interferon gamma (IFN-y), Ferritin, and D-Dimer using the Randox Evidence Multi-STAT analyser.

In group two we measured cytokines in matched postmortem serum and VH samples from decedents who tested positive for either fentanyl (n=10) or fentanyl plus xylazine (n=6). A negative control consisted of samples negative for drugs of abuse (n=9).

In group three we measured cytokines in oral fluid (OF) of living individuals who tested positive for either fentanyl (n=10) or fentanyl plus xylazine (n=10). A negative control consisted of samples negative for drugs of abuse (n=10).

For group two and three, we measured the concentrations of IL-6, IL-10, IL-1β, and TNF-α using ELISA kits purchased from ThermoFisher.

Results: Group one: Our findings revealed significant elevations in IL1-β, IL-6, and TNF-α in VH of individuals positive for fentanyl relative to the control. Cocaine-positive subjects showed marked increases in IFN-y and Ferritin. Interestingly, no significant variations were observed in the methamphetamine/amphetamine-positive group across the tested markers.

Group two: Concentrations of IL-6 were markedly higher than the other cytokines and over half of the serum and VH samples exceeded the upper limit of the calibration range. However, there were no significant differences in serum or VH cytokine concentrations for fentanyl or fentanyl plus xylazine relative to the negative control. None of the VH samples tested in this group were positive for IL-1β.

Group three: IL-10 and IL-6 were measured in all but two OF samples. In contrast, IL-1β and TNF-α were only measured in 11 of the samples. There were no significant differences in OF cytokine concentrations for fentanyl or fentanyl plus xylazine relative to the negative control.

Discussion: Our research aimed to discern whether the presence of cocaine, amphetamines, fentanyl, or fentanyl plus xylazine influences inflammation profiles in deceased and living individuals. We had a unique focus on VH to evaluate its effectiveness in reflecting drug-induced inflammatory profiles. Significant measures were taken when comparing fentanyl to our negative control, but we had conflicting results between groups. A sub-focus on fentanyl and xylazine showed no significant differences relative to negative controls in any of the matrices we tested.

This study enhances our understanding of the specific inflammatory profiles associated with different drug intoxications and their impact on ocular physiology. Herein we advance forensic toxicology by demonstrating the utility of VH for detecting drug-induced inflammatory changes. Our findings offer crucial insights into the ocular impact of these drugs, informing both clinical treatments for substance abuse and public health strategies. Further research is required to assess how OF can aid in this space.

New York State Police's Evidential Oral Fluid Testing Pilot Program

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Introduction: The use of oral fluid for drug testing is applicable in many sectors of forensic toxicology, including workplace drug testing, pain management, and impaired driving. It has many advantages for driving under the influence of drugs (DUID) investigations, but its widespread implementation in the United States has been hindered by the need for legislative action in many states, as well as a lack of laboratory resources available to develop and validate the testing methods.

In the United States, blood and urine are the specimens most commonly collected for DUID. In New York, there are numerous challenges to obtaining blood draws which lead to long delays in collection, or the inability to collect it, and the use of urine in DUID cases is discouraged. Since New York has had saliva in its impaired driving statute for decades, it provides a viable alternative.

Objectives: In September 2023, the New York State Police (NYSP) launched an evidential oral fluid pilot program to allow stakeholders to adapt to the alternate specimen type, identify any hurdles to broader implementation, and to collect blood and oral fluid correlation data. Once the pilot program was completed, the focus for the NYSP evidential oral fluid program is to collect blood and oral fluid in all serious injury and fatal motor vehicle investigations, and to have oral fluid be a stand-alone option for non-injury misdemeanor DUID investigations.

Methods: The Quantisal™ oral fluid kit was used for specimen collection. The oral fluid testing scheme meets or exceeds the National Safety Council's Alcohol, Drugs and Impairment Division's recommendations for Tier 1 drugs[1] plus phencyclidine (PCP) and delta-8-tetrahydrocannabinol (THC). Screening for all compounds is accomplished using a liquid-liquid extraction followed by liquid chromatography tandem mass spectrometry (LC-MS/MS) analysis. Drug confirmation is accomplished using solid phase extraction (SPE) and LC-MS/MS. One confirmation targets delta-9-THC and delta-8-THC; the other method targets the remaining 30 drugs and metabolites.

Blood and urine drug testing also meets or exceeds the recommendations for Tier 1 drugs plus phencyclidine (PCP). The protocol screens for 14 drug/drug classes using enzyme linked immunosorbent assay (ELISA). Drug confirmations use SPE combined with various gas chromatography/mass spectrometry (GC-MS) and LC-MS/MS analyses.

During the pilot program, NYSP Drug Recognition Experts (DREs) were instructed to collect oral fluid in addition to blood in routine non-injury DUID investigations.

Results: The pilot program ran from September 2023 through May 2024, and the laboratory received 243 oral fluid samples; 200 blood-oral fluid pairs, 11 urine-oral fluid pairs, and 32 oral fluid only samples were submitted. Testing was completed for 238 oral fluid samples and the most detected drugs were delta-9-THC (55%), cocaine/benzoylecgonine (41%), fentanyl (32%), and methamphetamine/amphetamine (31%).

As of July 31, 2024, testing was completed for 196 blood-oral fluid pairs. There was an overall correlation rate of 97% in which at least one drug matched in both samples; 56% had an exact match for parent and/or metabolite; oral fluid detected additional drug(s) in 40% of pairs. The most common additional drugs detected in oral fluid included cocaine (22), 6-acetylmorphine (18), morphine (16), delta-9-THC (12), methamphetamine (11),

amphetamine (10), and fentanyl (10).

Discussion: Blood-oral fluid paired samples showed an excellent overall correlation of 97%. Detection differences were primarily around cut-offs. Some of the drugs reportable only in the oral fluid did show elevated blood ELISA screens, but there was no trend noted related to concentration or collection times. This is likely due to drug presence below the ELISA blood assay cut-off.

Feedback from the DREs involved in the pilot program was very favorable. The process was fast and easy. Challenges noted were that the indicator did not turn blue, or the subject did not follow directions (chewed on pad, removed collector).

[1] "Recommendations for the Toxicological Investigation of Drug-Impaired Driving and Motor Vehicle Fatalities – 2021 Update," A. D'Orazio, A. Mohr, A. Chan-Hosokawa, C. Harper, M. Huestis. J. Limoges, A. Miles, C. Scarneo, S. Kerrigan, L. Liddicoat, K. Scott, B. Logan,. *J Anal Tox*, 2021, 45:529-536.

S14

Changes in blood cannabinoid concentrations over multiple collection times in driving under the influence of drug(s) casework

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Introduction: Forensic toxicologists are often asked to interpret cannabinoid results, including to provide information on time of marijuana use in DUI cases. However, Δ9-Tetrahydrocannabinol (THC) and its metabolites, 11-hydroxy-THC (11-OH-THC) and 11-nor-9-carboxy THC (THC-COOH), have been demonstrated to be detectable in blood for days after last use in chronic users in research studies. In driving under the influence of drug(s) (DUID) casework, there is typically no reliable information regarding route of administration, history of use, or time of last use.

Objectives: Changes in blood cannabinoid concentrations across multiple time points in DUID casework will be evaluated. Specifically, THC, 11-OH-THC, and THC-COOH concentrations will be reviewed.

Methods: Cases selected for inclusion were submitted between January 2019 and January 2024 to NMS Labs by a specific state with multiple blood draws over time from a subject for a single incident and had a cannabinoid confirmation test performed. Confirmatory analysis was conducted by Liquid Chromatography/Tandem Mass Spectrometry (LC-MS/MS) with the following analytical measurement ranges in blood (ng/mL): THC (0.5 -50), THC-COOH (5.0-500) and 11-OH-THC (1.0-50). Concentrations were reported to 2 significant figures.

Results: Thirty-five (35) cases were identified that met the inclusion criteria and had all cannabinoid confirmation testing performed in blood with at least one sample yielding a reportable THC result. Cases contained either two (n=26), three (n=7) or four (n=2) samples. The time between incident and blood collection ranged from 00:32 hours to 12:42 hours. THC was identified in 81 samples, and concentrations ranged from 0.75 – 40 ng/mL. Six hours after the incident, 10 out of 17 samples had a THC concentration exceeding 5 ng/mL. 11-OH-THC was detected in 60 samples, with concentrations ranging from 1.0 – 16 ng/mL. THC-COOH was detected in 81 samples, at concentrations ranging from 7.1 – 470 ng/mL. Table 1 demonstrates the increase or decrease in cannabinoid concentrations over multiple blood draws. There were 46 total time point changes; 11-OH-THC fell below the reporting limit (n=14) or had no change in concentration (n=1), while THC had no change in concentration (n=2) for some time points.

Table 1.

	Increase in concentration at later time point			Decrease in concentration at later time point		
	n	Percentage increase from initial result (%)	Elapsed time since incident (hh:mm)	n	Percentage decrease from initial result (%)	Elapsed time since incident (hh:mm)
THC	11	7.0 - 37	02:12 – 06:10	33	5.0 - 65	02:16 – 12:42
11-OH-THC	6	3.2 - 34	02:12 – 06:10	25	3.9 - 64	02:47 – 08:52
THC-COOH	6	3.1 - 40	02:47 – 12:42	40	5.3 - 57	02:12 – 10:06

Discussion: For this study, cases had multiple blood draws submitted, often due to exigent blood draw samples and blood drawn with a warrant. It is assumed that the subject was under surveillance over the time course of blood draws. THC and the active metabolite 11-OH-THC were detected in blood specimens obtained up to 12 and 8 hours after incident, respectively. THC concentrations did not exhibit a classic rapid and/or constant decrease over time for most cases. This study underscores the difficulty in drawing conclusions and providing interpretation regarding time of marijuana use, even when multiple samples are obtained over time from a single incident.

S15

A Ketamine Trilogy: A Review of Three Different DUID Cases with the Same Driver.

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Introduction: Ketamine is a dissociative anesthetic agent first introduced in the 1960s as an alternative to PCP in attempt to create a safer anesthetic for use in both human and veterinary medicine. Ketamine's clinical utility has expanded beyond anesthesia due to its effective sedation and analgesic properties while maintaining cardiorespiratory stability. These pharmacological properties have led to ketamine use by emergency medical services at the roadside and as a newer therapy for resistant depression. As ketamine's popularity increases, the potential for abuse also increases. Recreational use of ketamine may be due to its hallucinogenic, analgesic, and dissociative properties. The potential adverse effects on driving including confusion, impaired memory, blurred vision, sedation, and visual or auditory hallucinations make ketamine a public safety matter.

Objectives: This presentation summarizes impairment observed in three different DUI investigations of the same person within a 6-month span. Ketamine was identified in biological specimens (one urine and two blood specimens) from all three incidents where there was no known medical administration. Observations gathered from police reports and body worn cameras are compared to the general impairment indicators expected from ketamine pharmacology.

Methods: The urine specimen submitted was screened by an eleven-panel ELISA (Dynex DSX, Neogen Assay Kits) and a liquid-liquid extraction (LLE) for base drugs with full scan GC-MS (Agilent 6890/5973C) after an evidential breath alcohol test (CMI Intoxilyzer 8000). Drug confirmation was conducted using a LLE with full scan GC-MS (Agilent 6890/5973C). The blood specimens underwent volatile analysis using HS-GC-FID/MS (Agilent 7890A/5975C) and were screened for drugs using an eleven-panel ELISA and a LLE with full scan GC-MS (Agilent 7890A/5975C). Ketamine was confirmed using a LLE with full scan GC-MS (Agilent 7890A/5975C) and quantitated using a GC-NPD (Agilent 6890N). Amphetamine was confirmed and quantitated by LC-MSMS (Sciex 3200 Qtrap). Cannabinoids were confirmed and quantitated by LC-MSMS (Sciex 5500+). Confirmation testing did not include norketamine in blood or urine.

Results: The urine specimen collected on 4/13/23 was positive for amphetamine, ketamine, and dehydronorketamine. The blood specimen collected on 6/26/23 was positive for amphetamine at 45 ng/mL, ketamine at 326 ng/mL, delta-9-THC at 1.0 ng/mL, hydroxy-THC at less than 1.0 ng/mL, and carboxy-THC at less than 5.0 ng/mL. The blood specimen collected on 9/23/23 was positive for ketamine at 1,174 ng/mL and amphetamine at 64 ng/mL.

Discussion: The driver underwent a DUI investigation three separate times. Two were due to collisions and one was provoked by the subject interacting with officers directing traffic caused by an unrelated accident. The driver had a history of severe anxiety and stated he was taking Adderall. Field sobriety tasks (FSTs) were only fully completed one time. During the FSTs the driver had HGN, VGN, LOC, poor balance on the walk and turn and one leg stand, and did not complete the finger to nose task properly. General observations that were common throughout the three incidents were lack of coordination and balance, disorientation, confusion, inability to complete sentences or thoughts, slurred speech, drowsiness, increased heart rate and blood pressure, and dissociation. These observations correlate well with the indicators for dissociative anesthetics captured in the DRE matrix. Both CNS depressants and dissociative anesthetics (DA) may present the same indicators for HGN, VGN, and LOC. Physiological indicators that differentiate DA are elevated pulse rate and blood pressure which in these cases may have been enhanced by concurrent use of a stimulant, and in one instance, cannabis.

Results from the 2024 Survey for Drug Testing in DUID and Motor Vehicle Fatality Investigations

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Introduction: In 2004, the National Safety Council Committee on Alcohol and Other Drugs (NSC-COAD; later the NSC Alcohol Drugs and Impairment Division (ADID)) set out to gain more information from toxicology laboratories on testing practices associated with driving under the influence of drugs (DUID) case testing in laboratories throughout the United States. Information on laboratory testing and capabilities, scope and cutoffs, and most frequently encountered analytes in DUID and motor vehicle fatality cases were gathered and reviewed to create a set of standardized recommendations for laboratories performing this type of casework. The first set of recommendations was published in 2007, followed by subsequent updates to the recommendations in 2013, 2017, and 2021.

Objectives: In 2024 the NSC-ADID undertook a review of the 2021 recommendations to update the recommendations document based on survey data results from laboratories performing DUID casework. The focus remains on assessing the Tier I and Tier II testing scope and cutoffs for screening and confirmation in blood and oral fluid.

Methods: A total of 229 laboratory directors or employees throughout the United States and Canada were contacted for survey participation via SurveyMonkey®. A total of 80 laboratories completed the survey. Questions related to laboratory testing and capabilities, scope and cutoffs for screening and confirmation, and compliance with the 2021 recommendations were the main focus of the survey. Additional questions regarding the most frequently encountered analytes, testing practices, and resource needs were also included.

Results: Of the 80 participating laboratories, 96% test blood samples, 68% urine samples, and 5% oral fluid. The top three screening methods for blood samples were Enzyme-Linked Immuno-Sorbent Assay (ELISA) (46%), Liquid Chromatography-Mass Spectrometry (LC-MS) (41%), and Liquid Chromatography-High Resolution Mass Spectrometry (LC-HRMS) (33%). The top three confirmation methods were Liquid Chromatography-Mass Spectrometry (LC-MS) (91%), Gas Chromatography-Mass Spectrometry (GC-MS) (69%), and Gas Chromatography-Flame Ionization Detection (GC-FID) and Liquid Chromatography-High Resolution Mass Spectrometry (LC-HRMS) (15%). The top three priorities for additional resources were increasing staffing and upgraded/new facility, training, and instruments for confirmation.

For screening practices in blood, 71-99% (mean=93%, median=94%) of laboratories screen for individual drugs in the Tier I scope, and 64-97% (mean=86%, median=88%) met the recommended screening threshold. For screening thresholds that changed in the 2021 recommendations, compliance ranged between 75-97%. The highest rate of compliance was for meprobamate, while the lowest rate was for 7-aminoclonazepam. For confirmatory practices in blood, 64-97% (mean=89%, median=91%) of laboratories confirm individual drugs in the Tier I scope, and 40-97% (mean=86%, median=89%) met the recommended confirmation threshold. For confirmation thresholds that changed in the 2021 recommendations, compliance ranged between 80-95%. The highest rate of compliance was for clonazepam, while the lowest rate was for buprenorphine. A total of 16% of laboratories met or exceeded all of the 2021 recommendations for confirmatory testing, while 47% are currently changing methods to meet the recommendations.

The top ten most prevalent drugs reported by laboratories in blood were THC and metabolites; amphetamine/methamphetamine; fentanyl; cocaine and metabolites; alprazolam/alpha-hydroxyalprazolam; clonazepam/7-aminoclonazepam; diphenhydramine; oxycodone; diazepam/ nordiazepam; hydrocodone. The top three most requested drugs for inclusion in Tier I due to increased prevalence were gabapentin, delta-8 THC, and novel benzodiazepines. Some drugs were recommended for removal from Tier I including meprobamate and carisoprodol.

Discussion: An updated manuscript of the recommendations is planned for the *Journal of Analytical Toxicology* following a critical review of the survey data and consideration of changing drug trends since the 2021 recommendations.

Driving under influence of delta8-tetrahydrocanabinol cases in Houston, Texas

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Introduction: There is growing interest in delta8-tetrahydrocanabinol (delta8-THC) as an alternative to delta9-THC, due to the passage of the 2018 US Farm Bill and the lack of uniform federal and state regulations. Delta8-THC is a structural isomer of delta9-THC with comparable though weaker psychoactive effects (67%)[1]. Currently, there is limited information on its prevalence and physiological and impairing effects.

Objectives: The purpose of this study was to evaluate the use of delta8-THC among drivers in the Houston area between September 2020 and May 2024 by reviewing driving while intoxicated (DWI) cases submitted to the Houston Forensic Science Center (HFSC). Toxicology test results, demographics, and case information were examined for the DWI cases that were tested positive for delta8-THC in blood.

Methods: Blood samples from the DWI cases were screened by enzyme-linked immunosorbent assay (ELISA). Presumptive positive cases for cannabinoids were confirmed for the presence of 11-hydroxy-THC (11-OH-THC), 11-nor-9-carboxy-THC (THC-COOH), cannabidiol (CBD), delta9-THC, and delta8-THC using validated liquid-liquid extraction and liquid chromatography-tandem mass spectrometry (LC-MS/MS) methods. The calibration range for these analytes was 1-100ng/mL (5-250ng/mL for THC-COOH). Behavioral and physiological information was obtained from the police reports. Limitations of this study include missing cases that were outsourced to an external forensic laboratory, which did not include delta8-THC in their analytical scope and having a limited number of cases that only tested positive for delta8-THC.

Results: During this period, 50% of confirmation cases were positive for cannabinoids. Of those cases, 2.4% (n=14) were positive for delta8-THC. Delta8-THC concentrations ranged from 1.1 to 120ng/mL (median 1.8ng/mL). All but one driver had at least one drug other than delta8-THC. Delta9-THC was found in 10 drivers ranging from 1.2 to 23ng/mL (median 4.2ng/mL). THC-COOH (24-130ng/mL) was found in 4 drivers and was unsuitable to report due to coeluting interferences in 6 drivers. 11-OH-THC was unsuitable to report in 7 drivers, and 1 driver had CBD. The most common drug classes other than cannabinoids found concurrently with delta8-THC were benzodiazepines and opioids, positive in 7 and 6 drivers respectively. Other drugs found in driver(s) were amphetamine/methamphetamine (4 drivers), ethanol, carisoprodol, and benzoylecgonine (3 drivers), and meprobamate, cocaine, and phencyclidine (1 driver). Demographic characteristics included an age range of 20-46 years old (average 34 years), and cases were 78% male including 1 Asian, 6 Black, and 7 White drivers.

Three cases of particular interest will be described in more detail. *Case 1*: 30YO Black male found asleep at the wheel with slurred speech, poor balance, and red, bloodshot eyes. Blood concentrations were delta8-THC 2ng/mL, delta9-THC 8.4ng/mL, THC-COOH 130ng/mL, and 11-OH-THC unsuitable to report. *Case 2*: 29YO White male found asleep and confused at the wheel with red, glassy, bloodshot eyes. Blood concentrations were delta8-THC 120ng/mL, delta9-THC 23ng/mL, and 11-OH-THC and THC-COOH unsuitable to report. *Case 3:* 30YO Black male reported to be asleep at the wheel then evaded arrest when police arrived. He was noted to have red, glassy, bloodshot eyes, slurred speech, sway, and a dazed and disoriented demeanor. Blood was positive only for delta8-THC at 1.2ng/mL.

Discussion: The HFSC data illustrated that delta8-THC will most likely be taken in combination with other drugs. The results for delta8-THC indicate the finding of 120ng/mL to be an abnormally high concentration. Intoxication symptoms from limited research for delta8-THC included lethargy, impaired coordination, slurred speech, and sedation which is consistent with police reports from the 14 DWI cases. It is important to share these findings to better understand the associated behavioral effects and how they affect driving.

[1] Kruger J, Kruger D. Delta-8-THC: Delta-9-THC's nicer younger sibling? *J Cannabis Res* 2022;4:4. https://doi.org/10.1186/s42238-021-00115-8

S18

Evaluation of Securetec DrugWipe® S as an Approved Roadside Oral Fluid Device in Alabama

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Introduction: With the alarming rise in drug-impaired driving incidents throughout the United States, law enforcement agencies are increasingly relying on roadside oral fluid (OF) devices as valuable tools for detecting drug impairment among drivers. These devices play a pivotal role in ensuring road safety by enabling swift and efficient identification of potential drug use by individuals suspected of driving under the influence of drugs (DUID), thereby mitigating the risks associated with drug-related accidents and fatalities. Alabama has previously approved three roadside OF devices for use throughout the state: Draeger DT5000, Abbott SoToxa, and the Randox MultiStat. In light of their critical importance in modern law enforcement practices, this study endeavors to undertake a comprehensive analysis of a new roadside OF device, the Securetec DrugWipe® S.

Objective: To evaluate the Securetec DrugWipe® S for use as a new roadside OF device for the state of Alabama.

Methods: The DrugWipe® S cartridge tests for five drugs or drug classes: cocaine, Δ-9-tetrahydrocannabinol (THC), opiates, amphetamine, and methamphetamine. The 6S cartridge also includes a test for benzodiazepines. The THC concentration cutoffs differ between the 5S and 6S, with the 5S having a cutoff of 5 ng/mL and the 6S having a cutoff of 10 ng/mL. In 2019, 55 volunteers in drug rehabilitation were evaluated in Jacksonville, Florida using the Securetec DrugWipe® 6S cartridges by the Alabama Department of Forensic Sciences (ADFS) in coordination with the Alabama Drug Recognition Expert (DRE) program. Additionally, a combination of confirmation samples (i.e., Quantisal oral fluid) from the 2019 Jacksonville study and simulated case samples were analyzed. For simulated samples, drug-free oral fluid was collected from volunteers, pooled, then spiked with known drug concentrations 50% above the concentration cutoff, at the concentration cutoff, and 50% below the concentration cutoff and analyzed on both the 5S and 6S cartridges 10 times at each concentration. Six negative and seven mixed positive controls at various concentrations were also tested. All reported results were evaluated using the Securetec WipeAlyser. The DrugWipe® S test was administered following standardized protocols, and positive results were confirmed through laboratory analysis. Performance parameters including sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and accuracy were calculated.

Results: The DrugWipe® S tests demonstrated suitable performance for THC and cocaine in authentic samples, with all parameters exceeding 80%. However, the availability of authentic positive samples for opiates, benzodiazepines, amphetamine, and methamphetamine was insufficient to draw conclusive results for these targets. To address this limitation, simulated samples were analyzed across all targets, yielding an overall performance criteria greater than 95%. In simulated samples, cocaine, opiates, and benzodiazepines demonstrated exceptional accuracy and sensitivity, each achieving 100%. NPV ranged from 89% to 100%, underscoring the reliability in correctly identifying true negatives. PPV exceeded 100% for all targets, indicating a high level of confidence in correctly identifying true positives.

Discussion: This study highlights the role of roadside OF as another tool for law enforcement to assist with DUID investigations. The evaluation of the DrugWipe® S, alongside established devices, reaffirms their significance in law enforcement DUID investigation practices. With the performance demonstrated for all targets, the DrugWipe® S exhibits robust capabilities in accurately identifying potential drug use in individuals suspected of DUID, thus contributing to enhanced road safety measures. While these findings demonstrate high sensitivity, specificity, and accuracy rates for both authentic and simulated samples, slight variations observed in certain parameters (specificity, sensitivity, and accuracy) may be attributed to metabolite cross-reactivity in authentic samples. Nonetheless, the comprehensive analysis conducted proved this device fit for purpose for use in roadside oral fluid drug testing, empowering law enforcement agencies to effectively detect drug use and mitigate associated risks on our roadways.

Application of LC-HRMS Screening to Blood DUID Cases: Benefits & Limitations

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Introduction: Liquid chromatography with high resolution mass spectrometry (LC-HRMS) has become a well-established technique that can be used for both screening and confirmation of a wide range of substances.

Objectives: To develop and validate a blood LC-HRMS screening and confirmation method that meets the following objectives: small sample volume, rapid sample preparation, and sufficient sensitivity to meet the confirmation concentrations for Tier I compounds in the 2021 NSC-ADID Recommendations as well as incorporate a wide range of other drugs and metabolites, including psilocin. Apply the method to authentic casework and compare to previous testing methods.

Methods: The developed LC-HRMS method required 0.2 mL of blood specimen and simple protein precipitation with 0.6mL of cold acetonitrile. Ascorbic acid, 10 µL of 0.01M, was added prior to the sample and again prior to evaporation of solvent to stabilize free psilocin. Acidified methanol was also added prior to evaporation to aid in the recovery of volatile bases (e.g., amphetamine). LC-HRMS analysis was conducted on a Thermo Vanquish liquid chromatograph (LC) with a Thermo QExactive HRMS run in full MS mode with data-dependent fragmentation using a targeted inclusion list. A gradient LC program with a Restek Raptor Biphenyl 2.7µm (50 x 2.1 mm) column was used for both positive and negative modes (separate injections). Method validation included experiments for selectivity, sensitivity, carryover, processed sample stability, robustness and a case comparison. Ionization suppression/enhancement was not conducted, rather sensitivity was evaluated in five different matrix sources. Validation was completed for 262 substances.

For the method comparison, screening was conducted using a basic extraction with scan GC-MS and an 11 panel enzyme linked immunosorbant assay (ELISA). For cases tested by LC-HRMS, a 3 panel ELISA was also performed as described below.

Results: The sensitivity of the method met or exceeded the recommended confirmation cutoff concentrations for 33/36 Tier I substances and included Tier II substances with the exception of inhalants, GHB, and synthetic cannabinoids. Cannabinoids were not able to be detected at the Tier I confirmation concentrations. The LC-HRMS limit of detection for buprenorphine and norbuprenorphine was just 0.5 and 1 ng/mL, respectively. ELISA screening was therefore maintained for buprenorphine and cannabinoids. Due to past utility of the ELISA benzodiazepines screen to detect designer substances and the use of a targeted inclusion list for the LC-HRMS, the ELISA benzodiazepines panel was also maintained.

The drug testing results of 58 blood impaired driving cases before the LC-HRMS screen was implemented and 58 different cases after were compared. The results are summarized in the table below.

Condition	*n*	Unique substances	Total Identifications	Identifications / positive case	None Detected Cases
Prior to LC-HRMS	58	38	152	3.9	19
After LC-HRMS	58	60	216	5.4	16

Discussion: The developed LC-HRMS method met the primary goals of low specimen volume, simple sample preparation, and overall excellent sensitivity for a wide range of substances, including those in Tier I and II of the recommendations. A limitation of the method was poor sensitivity for cannabinoids. After implementing LC-HRMS screening, the number of unique substances (drugs and/or metabolites) increased by 58%, the number of identifications by 42% and the number of substances identified in each positive case by 36%. Although the same number of cases were tested before and after, the comparison was limited as none of the same cases were tested by both methods. A selection of individual case reports shall also be discussed to highlight the utility of incorporating LC-HRMS in routine screening of blood impaired driving cases.

S20

Pulse testing of negative federally regulated urine specimens by LC-QTOF-MS indicates use of additional impairing substances

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Introduction: The urine drug testing panel for federally regulated specimens in the United States comprises fourteen schedule I and II analytes. In an effort to subvert the testing process and avoid detection of drug use, employees in safety-sensitive positions may use impairing substances that are not on the authorized testing panel or substitute their sample with synthetic urine products.

Objectives: Determine the prevalence of synthetic urine products and drugs outside the current testing panel by pulse testing of negative urine specimens using Liquid Chromatography Quadrupole Time-of-Flight Mass Spectrometry (LC-QTOF-MS).

Methods: De-identified, negative, federally regulated urine specimens (n=3,714) were obtained from Department of Health and Human Services-certified (HHS-certified) laboratories. Samples were prepared via protein crash with two parts methanol, followed by LC-QTOF analysis using a Restek Raptor biphenyl column, ammonium formate and methanol with formic acid mobile phases, and data independent acquisition on a Waters Xevo G2-XS using alternating low and high collision energy scans (MS^E). The scope currently encompasses 73 drugs and metabolites, including 21 opioids, 12 benzodiazepines, 7 stimulants, 4 hallucinogens, and 4 sedatives, as well as 3 lifestyle markers. Potential use of synthetic urine products was identified by the absence of three features with masses of 184.12, 228.15, and 264.11 only present in authentic urine samples. The structures of the features were not elucidated. Drug reference materials were analyzed alongside the specimens to assure identification.

Results: Benzoylecgonine at concentrations below the program initial test cutoff of 150 ng/mL was identified in 0.81%% (n=30) of samples, indicating that positivity could be increased if the program cutoff was lowered. Seventeen analytes outside the current testing panel were identified. Common findings included diphenhydramine (3.15%, n=117), gabapentin (1.40%, n=52), o-desmethyltramadol (0.62%, n=23) and mitragynine (0.43%, n=16). These results can be compared to the prevalence of the most prevalent drug in Federal testing, Δ9-THC carboxy metabolite (0.92% in 2023) and to a total drug positivity rate of 2.43% in Federal specimens in 2023. Synthetic urine products were identified in 2.86% of evaluated specimens (91/3,178).

Discussion: The results illustrate three different ways pulse testing with LC-QTOF-MS continues to prove useful to the Federal workplace drug testing program by providing data on the prevalence of potentially impairing drugs outside the program panel, the prevalence of urine drug test subversion, and by demonstrating possible effects of cutoff changes.

Evaluation of Sevoflurane Interference with Alcohol Reporting, Including a Potential Authentic Case Sample

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Introduction: Sevoflurane (fluoromethyl 1,1,1,3,3,3–hexafluoroisopropyl ether) is a halogenated volatile anesthetic that is routinely used in clinical settings. Sevoflurane is typically inhaled, and it acts on the central nervous system to affect pain perception (Campagna et al. N Engl J Med. 2003;348;2110-24). The Houston Forensic Science Center (HFSC) Toxicology Section performed an interference study on sevoflurane according to quality assurance recommendations from the December 2023 Robert F. Borkenstein Course on Alcohol and Highway Safety and documented sevoflurane studies (Tiscione et al. J Anal Toxicol. 2011;35:501-511).

Objectives: The goal of this study was to determine whether sevoflurane, if present, could interfere with the reporting of ethanol, methanol, isopropanol, or acetone using the HFSC volatiles analysis method.

Methods: Sevoflurane in deionized water (0.1%) (Tiscione, 2011) was used to fortify ethanol samples at various concentrations in triplicate that spanned the analytical range of the HFSC volatiles method, including the legal limit (0.08 g/100 mL) and an approximation of the enhanced DWI level within the jurisdiction of HFSC stakeholders (0.16 g/100 mL). Theoretical sevoflurane concentrations spanned 0.0013-0.0127 g/100 mL (10, 25, 50, 75, and 100 µL aliquots of 0.1% sevoflurane solution). Samples were analyzed using dual-column (Restek 30 m x 320 µm id x 1.8 µm (BAC1) or 0.6 µm (BAC2)) headspace gas chromatography with flame ionization detection. Acceptance criteria were monitored in adherence with the HFSC Analytical Manual, including peak to valley ratios ≥10 and %difference between individual FID concentrations and the average concentration within 5 or 10%.

Results: Sevoflurane did not affect methanol, isopropanol, or acetone quantification as evidenced by clear separation in retention times on both analytical columns. Visually, a shoulder peak on the ethanol peak was consistently observed on one detector in every sample, while sevoflurane and ethanol co-eluted on the other. Ethanol targets at 0.02 and 0.08 g/100 mL with all sevoflurane concentrations consistently failed to meet ethanol reporting acceptance criteria. Ethanol targets at 0.16 and 0.40 g/100 mL with 0.0013 and 0.0034 g/100 mL sevoflurane caused slightly elevated ethanol concentrations, and all reporting acceptance criteria were met; however, the ethanol result remained within the method's uncertainty of measurement (UM, 9.4%). The same ethanol targets coupled with all other sevoflurane concentrations caused failed acceptance criteria, preventing the reporting of positive ethanol results.

One potential authentic sevoflurane case was identified in recent HFSC casework. Upon first volatiles analysis, a shoulder peak was visually identified on one detector's ethanol peak, consistent with the experimental sevoflurane samples, and acceptance criteria were not met to report the ethanol result, prompting re-analysis. Upon re-analysis, the interference was no longer observed, the case sample met acceptance criteria, and the ethanol result was therefore reported. Case details and chromatographic data will be shared.

Discussion: This study demonstrated that sevoflurane is potentially an interferant with the HFSC ethanol analysis. However, adhering to current acceptance criteria is sufficient to monitor for sevoflurane interference. In the few situations where sevoflurane was present and ethanol reporting acceptance criteria were met, the ethanol quantification remained within the method's UM.

The authentic case sample showed that adhering to HFSC alcohol reporting criteria allowed for successful analysis of a potential sevoflurane interference case. Initial failed acceptance criteria prompted re-analysis, upon which all acceptance criteria were met, and ethanol results were reported. Sevoflurane, being highly volatile, potentially evaporated prior to re-analysis (Kovatsi et al. J.Sep. Sci.; 2011;34;1004-1010); evaluation of sevoflurane sample stability is currently in progress. If upon re-analysis acceptance criteria had again failed, ethanol results would not have been reported, and the issued report would have declared the sample unsuitable for analysis.

S22

Current trends in psilocin casework

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Introduction: Psilocin is a serotonin 2A receptor agonist that is typically introduced into the body by the consumption of "magic mushrooms" which contain psilocybin as a phosphorylated precursor drug. This potent hallucinogen produces psychedelic effects for those who are seeking a recreational experience or possible treatment for depression. Although psilocybin is a Schedule 1 substance, several cities and states have decriminalized the compound to allow psilocin for medicinal uses, beginning with Oregon in 2020. Due to a rise in specific requests for analysis, AFMES implemented an updated LC-MS/MS method for high-throughput screening and confirmation for psilocin in forensic toxicology specimens.

Objectives: After attending this presentation, audience members will learn about trends in psilocin positive casework over the last 3 years.

Methods: Samples were analyzed by similar but separate LC-MS/MS protocols for both the screening and confirmation methods, where the screening method used an Agilent Poroshell 120 phenyl hexyl 2.7 µm, 2.1 x 100 mm column and the confirmation method used a Restek Raptor™ ARC-18 2.7 µm, 3.0 x 100 mm column. During development, interferences were noted between psilocin and the endogenous isomer known as bufotenine, so the chromatography was optimized to resolve between these substances.

Results: From the time the method went online in September 2021, the number of psilocin positive cases was very consistent, up until mid-year 2023 when the number of psilocin positives began to increase. Of these cases, the vast majority were investigative cases, with only four driving while intoxicated, one drug facilitated sexual assault, one aircraft incident, and two postmortem cases being reported. Although the method is reported qualitatively, the signal of psilocin in blood cases ranged from the limit of detection (LOD) of 1 ng/mL to approximately 100 times the LOD. The majority of blood cases reported signal intensity closer to LOD, which underscores the need for low limits of detection in blood specimens. In urine, the signal of psilocin in cases was more variable. Psilocin in urine cases was detected near the LOD of 1 ng/mL, as well as some cases having overloaded psilocin signals that needed a 1:200 dilution before acceptance criteria was met. The majority of cases were derived from coastal states as compared to interior states, with the highest density of psilocin positives originating from California each year.

Discussion: Due to an increasingly favorable perception of psilocin as a possible therapeutic agent for mental health, it has returned as a prevalent drug of abuse in forensic toxicology casework. Meanwhile information about the psychotic effects and depersonalization traits that emerge from chronic abuse is less widely shared and remains as uncommon knowledge. This species of mushroom is also more available through online retailers and is a renewable source if the spores are grown at home. The recent uptick in positive cases suggests that psilocin abuse is becoming more prominent, which underscores the usefulness of a method for psilocin analysis in the preservation of safety and readiness of military service members.

Analysis of Nutraceutical Products for Pharmacologically Active Substances

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Introduction: The term nutraceutical was coined in 1989 by Dr. Stephen DeFelice. Products considered nutraceuticals are derived from plant and/or food sources, with claims of therapeutic benefits. These products are part of a growing market of dietary supplements lacking Food and Drug Administration (FDA) regulation. Nootropics and cannabidiol (CBD) products exist under the umbrella of nutraceuticals. Nootropics are products often termed 'smart drugs' / 'cognitive enhancers'. Nootropics are formulated to target memory, productivity, stress, and other body functions. They are marketed aggressively to college students and consumers prescribed ADHD medication as better alternatives. CBD products are often marketed to treat pain, stress, anxiety, and insomnia. Many companies target first responders, promising zero delta 9- tetrahydrocannabinol (delta 9-THC).

Objectives: Analyze nootropic products marketed to youth and CBD products marketed toward first responders for chemical constituents and accuracy in labeled claims of pharmacologically active substances.

Methods: The following nootropic products were analyzed: truBrain, Thesis, Addall, Everyday Dose Mushroom Matcha+, and Thorne dietary supplements. The following CBD products were also tested: CBD OPS Broad spectrum oil, First Responder Fuel 10-8 Broad Spectrum PCR Gel Caps, CBD Topical Roll-On, 1000MG Broad Spectrum Tincture, and 1000MG Broad Spectrum Tincture- Lemon Flavor (Rescue 1 CBD), Daily balance 25mg CBD softgels, Reserve Softgels 25MG CBD, 2.5mg THC (+PlusCBD), Hemp Bombs CBD Capsules and Gummies, CBD Softgels and Full Spectrum CBD Softgels (Green Roads), and Bud Love Tropical Shipwreck, Berry Bhang, and Icy J.

Products were diluted to 400 µg/mL in methanol and analyzed using gas chromatography-mass spectrometry general screening method utilizing an HP-5MS Agilent column (30m, 0.250mm, 0.25 µm). CBD containing samples were further diluted to 4 µg/mL and analyzed using liquid chromatography tandem mass spectrometry with a validated method of 27 cannabinoids and their acetates using a Shim-Pack Velox C18 column (3.0 x 150 mm, 2.7 mm) for chromatographic separation. Concentrations of the cannabinoids were calculated using a calibration curve from 10 to 1000 mg/mL.

Products (gummy, softgel, or liquid aliquot) were analyzed for residual solvents and ethanol using a headspace gas chromatography dual flame ionization detector with RTZ-BAC PLUS 1 (30 m, 0.32 mmID, 1.8 µm df) and RTZ-BAC PLUS2 (30 m, 0.32 mmID, 0.6 µm df) columns. Residual solvents were checked from all three solvent classes using primary reference materials.

Results: Qualitative analysis revealed that not all ingredients listed on the nootropic packaging were present in the product. The Sleep formula from truBrain lists CBD as an ingredient; none was identified. The Flow formula does not list CBD on the ingredient list, even though it is labeled on the packaging. CBD was found in this product, showing inconsistency and confusion in labeling.

Three of the 13 products analyzed did not have labeled concentrations. Of the 10 CBD products tested with labeled concentrations, CBD OPS oil (n=3) and Hemp Bombs capsules (n=3) were determined to have a 12% difference versus the advertised CBD concentration. The remaining products ranged from 28% to 60% differences. Three products had detectable concentrations of delta-9-THC.

All products tested for residual solvents contained at least one residual solvent from the reference standards, including solvents such as methanol, ethanol, and 1,2-dichloroethane.

Discussion: Websites and social media platforms claim that their products are highly effective, safe, and reliable in preventing failed drug tests. However, the labels on the tested samples did not match the analytical results. Unregulated products containing undisclosed compounds and CBD products with delta-9-THC may cause adverse effects, potentially impacting health, safety, and drug test outcomes.

Analysis of Vaping Products Confiscated from Public School Systems Spanning Across the Commonwealth of Virginia

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Introduction: United States Food and Drug Administration (FDA) regulations prohibit the sale of electronic cigarettes (e-cigarettes) to consumers <21 years old and the sale of flavors other than menthol and tobacco in pod-based products to further discourage underage use. Despite FDA regulations, national survey data report continued e-cig use among middle and high school students, and that flavored products (such as fruity, candy, dessert) are the most commonly reported as used in under age populations. In addition to known safety concerns, such as lung injury, e-cigarettes have been adapted for discreet consumption of drugs other than nicotine (DOTNs), such as cannabidiol (CBD), Δ9-tetrahydrocannabinol (Δ9-THC), and other controlled and recreational substances. Current studies on adolescent e-cigarette use rely on self-reported data, with unknown accuracy and self-reporting biases. This study executed a novel approach, analyzing e-cig devices and their corresponding liquids which were confiscated directly from students in Virginia public schools.

Objectives: To evaluate vaping products confiscated from public school students across the Commonwealth of Virginia to determine chemical composition of their liquids and analyze vaping trends in adolescents.

Methods: This project was deemed as not human subjects research by the Virginia Commonwealth University (VCU) Institutional Review Board prior to study initiation. E-cigarette devices were confiscated from students by public school officials from 2022 to 2024. Information regarding student grade level, symptomatology, and device acquisition was requested. In total, 600 products from 10 school districts were submitted to the Laboratory of Forensic Toxicology Research in the Department of Forensic Science at Virginia Commonwealth University. After coding using REDCap, e-liquids were analyzed using gas chromatography-mass spectrometry,liquid chromatography-tandem mass spectrometry,and headspace-gas chromatography-dual flame ionization detection.

Results: In terms of product type, 88% were disposable, 8.2% were pod-based, 1.7% were refillable devices, and 2.2% were paraphernalia. Fruity flavor profiles were most common (53.5%), followed by dessert and candy-based profiles (42.8%), menthol (3.5%), and tobacco (0.2%). Most products were collected from grades 9-12 (66.5%), followed by grades 6-8 (5.5%), and grades <6 (0.5%); grade information was not available for 27.6% of products. Of the 224 samples chemically analyzed to date, the following compounds were identified: nicotine (80%), ethanol (12%), methanol (0.4%), one or more cannabinoid (20%), menthol (11%), one or more synthetic coolant (64%). Identified cannabinoids include tetrahydrocannabinol (Δ8, Δ9, Δ10, Δ6a,10a, exo), THC acetate (Δ8, Δ9), tetrahydrocannabivarin (Δ8, Δ9), hexahydrocannabinol, cannabidiol, cannabigerol, and cannabinol. Two paraphernalia samples (a pipe and “stash jar”) were determined to contain methamphetamine, nicotine, phytocannabinoids, and 5-fluoro BZO-POXIZI.

Discussion and Conclusion: A wide variety of commercially available products have been confiscated from school students, including elementary school populations, demonstrating that current legislation, policies, and/or code enforcement preventing underage sales of vaping products is insufficient to curb youth vaping. Compared to previous collection cycles, the prevalence of nicotine-based products decreased, while cannabinoid-based products increased, suggesting greater interest and/or access. Disposable products were the most common device type, likely due to their less stringent flavor regulations. Ethanol, additives, and a variety of flavoring chemicals were identified. The information gleaned from this study can support educational programs, policies, and legislation to protect communities and support health and safety programs.

S25

A Novel Screening Workflow for Nitazene Analogs using LC-QQQ Precursor Ion Scan Acquisition

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Introduction: Nitazene analogs have been relevant to the forensic toxicology community since 2019 with the appearance of isotonitazene, and these analogs continue to proliferate within the illicit drug market. With the emergence of new novel psychoactive substances (NPS) like nitazene analogs, accurate and reliable screening methods are crucial. However, more commonly used immunoassay screening methods can potentially produce false results due to lack of cross-reactivity and the lack of commercially available kits for nitazenes. To overcome these challenges, liquid chromatography quadrupole time-of-flight mass spectrometry (LC-QTOF-MS) has emerged as a favorite for NPS screening due to its ability to perform non-targeted screening with increased sensitivity. While effective, LC-QTOF-MS instrumentation comes with high capital and maintenance costs, steep learning curves, and time-consuming data processing, thereby limiting access to this instrumentation for some laboratories. Liquid chromatography triple quadrupole mass spectrometry (LC-QQQ) instrumentation is commonly utilized in forensic toxicology laboratories but is typically preferred for quantitative analysis using multiple reaction monitoring for increased sensitivity and specificity. However, LC-QQQ can be operated using precursor ion scan acquisition (an inverse of product ion scan acquisition) which allows scanning of possible precursor ions from targeted product ions at specified collision energies. This acquisition could allow laboratories to take advantage of analyte classes that have common fragmentation patterns, like nitazene analogs, where a set of shared characteristic fragments can be used to screen for a variety of parent compounds in a sample.

Objectives: The goal of this study was to develop and validate a novel screening method for nitazene analogs using LC-QQQ operated in precursor ion scan mode. Seven nitazene analogs (4'-OH nitazene, 5-methyl etodesnitazene, isotonitazene, metodesnitazene, N-piperidinyl etonitazene, N-pyrrolidino etonitazene, and protonitazene) and one internal standard (metodesnitazene-D4) were used to develop and validate this method.

Methods: A previously described liquid-liquid extraction (LLE) method was used for sample preparation. Briefly, 0.5 mL of blood was extracted using ammonium hydroxide, borate buffer, and 1-chlorobutane and reconstituted in mobile phase (5 mM ammonium formate and 0.1% formic acid in water and 0.1% formic acid in acetonitrile). For sample analysis, an Agilent 1290 Infinity II liquid chromatograph coupled to an Agilent 6475 Triple Quadrupole was operated in precursor ion scan mode and separation was achieved using gradient elution. This study optimized electrospray ionization source and MS acquisition parameters, while LC parameters were optimized in a previously published study. Agilent MassHunter Qualitative Analysis software was used for data analysis with a custom workflow.

Results: Five ions were monitored: *m/z* 72.1 and 100.1 (nitazenes with no substitutions at the R2 diethylamine position), *m/z* 98 and 112 (for pyrrolidino and piperidinyl ring substitutions, respectively), and *m/z* 104.1 for the internal standard. Values for collision energy, cell accelerator voltage, and scan time were optimized for each ion. Limit of detection (LOD) ranged from 1.0 ng/mL to 5.0 ng/mL, depending on the ion and the compound. Generally, *m/z* 100.1 demonstrated lower LODs than other ions. Ionization suppression/enhancement was calculated per ion and ranged from -54%-107%. Any interferences observed did not affect the detection of the ions at the LOD.

Discussion: Forensically relevant nitazene analogs are frequently changing, which makes it difficult for laboratories to constantly develop and validate updated methods. This study demonstrates how laboratories can utilize instruments that are likely already available in-house, saving time and money when screening for nitazene analogs. This precursor ion scan method established that monitoring just five product ions can rapidly detect nitazene analog precursors down to 1.0 ng/mL, with the potential to capture additional compounds. This method has the potential to give laboratories the flexibility to screen for a wide variety of nitazene analogs using a simple LLE workflow.

S26

Evaluation into Action - Validation of a Comprehensive 48-well Plate SLE Quantitative Method for all Tier I Compounds (and more) via LC/MS-MS after Laboratory Evaluation Using NHTSA's DUID tool.

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Introduction: The National Highway Traffic Safety Administration (NHTSA) developed the Drug-Impaired Driving Criminal Justice Evaluation Tool (DUID tool) to assist state, local, territorial, and tribal governments with assessing and strengthening their drug-impaired driving programs.[1] An evaluation of the Kansas Bureau of Investigation's (KBI) Toxicology Laboratory conducted using the DUID tool identified three areas in need of improvement: transition to quantitative reporting, meeting all 2021 Tier I cutoffs, and decreasing turnaround times. The laboratory currently utilizes five qualitative methods for the confirmation of Tier I compounds in whole blood, as defined by the National Safety Council's Alcohol, Drugs, and Impairment Division (2021 update[2]).

Objectives: Develop and validate a method to address the areas of improvement identified by the DUID tool evaluation. The proposed method utilized 48-well plates with confirmation and quantitation via LC/MS-MS for Tier I compounds, in addition to, confirmation and cutoff reporting for some Tier II compounds and other geographically relevant compounds.

Methods: A panel of 75 analytes including all Tier I compounds, regionally relevant Tier II compounds, and internal standards was selected for method development. In 48-well plates, 1 mL of whole blood was spiked with certified reference material and preconditioned with 1% NH_4OH. Samples were then loaded on to a Biotage® ISOLUTE SLE+ 1-mL 48-well extraction plate using Biotage® Extrahera™ automated extraction equipment. Samples were eluted with 4 mL of a 90:10 mixture of dichloromethane and isopropanol, followed by evaporation under nitrogen. Samples were reconstituted in 500 µL of mobile phase and analyzed on a Waters Xevo TQD coupled with a Waters Acquity I-Class UPLC. Chromatographic separation was obtained via a Waters CORTECS T3 column (2.1 x 75 mm, 1.6 µm), with mobile phases (A) 0.1% Formic Acid in H_20 and (B) Acetonitrile, over a 10.6 minute run in positive ion mode.

Results: This method was validated to ANSI/ASB Standard 036, Standard Practices for Method Validation in Forensic Toxicology (1st edition, 2019). The limit of detection (LOD) was set as the lowest calibrator for quantitative compounds and the decision point calibrator for qualitative compounds. The target limit of quantitation (LOQ) of 0.5 ng/mL could not be met for Buprenorphine and Norbuprenorphine. Instead, an LOQ of 1 ng/mL was set for these two compounds. Carryover and interferences were not observed for all compounds. Ion suppression ranged from -0.04% (Buprenorphine and Flualprazolam) to -67% (Sertraline). Fifteen compounds showed minor ion enhancement (≤ 5%). All matrix effects were compensated for by internal standards. Extracted sample stability was evaluated on the autosampler at 10°C for 72 hours. Compounds were relatively stable for 72 hours except for many benzodiazepines, which averaged 24 hours before their average signal fell below 20%.

Discussion: The KBI Toxicology Laboratory evaluated its practices and procedures using the NHTSA DUID tool, to identify areas in need of improvement. By using this tool, the laboratory was able to secure funding through NHTSA to support the validation of a quantitative method for all Tier I compounds and the qualitative confirmation of additional regionally relevant compounds. The new method will improve efficiency and throughput by reducing the number of confirmation methods employed by the laboratory and reducing turnaround time.

References:

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[2]D'Orazio, A. L., et, al. Recommendations for Toxicological Investigation of Drug-Impaired Driving and Motor Vehicle Fatalities—2021 Update. *Journal of Analytical Toxicology,* 45:529-536. doi:https://doi.org/10.1093/jat/bkab064

S27

Interference of exo-THC with delta9-THC in LC-MS/MS confirmation analysis

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Introduction: Exo-tetrahydrocannabinol (exo-THC; delta9,11-THC or delta11-THC) is a synthetic isomer of Δ9-tetrahydrocannabinol (delta9-THC). Exo-THC may have similar but less potent pharmacological effects than delta9-THC (Beardsley et al. J Pharmacol Exp 1987;241:521-6; Compton et al. J Med Chem 1991;34:3310-6). Structurally, exo-THC has a double bond outside of the cyclic ring at C11. Shortly after implementing a LC-MS/MS cannabinoids confirmation analysis in 2021, Houston Forensic Science Center (HFSC) noticed a significant interference with 11-nor-9-carboxy-delta9-THC (THC-COOH). During the revalidation process of the cannabinoids method to improve analyte separation, HFSC observed exo-THC eluting close to delta9-THC and its deuterated internal standard, delta9-THC-d3. This study brought awareness of a possible interference that could be misidentified as delta9-THC.

Objectives: The interference between the synthetic isomer, exo-THC, and delta9-THC and how HFSC addressed this interference were demonstrated. An exogenous interference study was performed to ensure the analytes of interest in the method were free from potential interferents. This study included tetrahydrocannabutol (THCB), tetrahydrocannabiphorol (THCP), 11-Hydroxy-delta-8-tetrahydrocannabinol, 11-Nor-9-carboxy-Δ8-tetrahydrocannabinol, cannabichromene (CBC), cannabinol (CBN), cannabigerol (CBG), cannabigerolic acid (CBGA), tetrehydrocannabinolic acid (THCAA), delta6-THC, delta10-THC and exo-THC.

Methods: The cannabinoids confirmatory analysis utilized Agilent 1290 Infinity II LC interfaced with 6470 MS/MS system using C18 column and quantified 11-OH-THC, CBD, THC-COOH, delta9-THC, and delta8-THC. The ion transitions were 318.2>123.0 and 318.2>135.0 for delta9-THC-d3 and 315.2>123.0 and 315.2>135.0 for delta9-THC. Retention times for delta9-THC-d3 and delta9-THC were approximately 6.587 and 6.616 minutes, respectively, within a 9-minute run time. Interference studies were performed analyzing blank blood samples fortified at 3ng/mL of delta9-THC and at 200ng/mL, 50ng/mL and 10ng/mL of exo-THC. Additional blood samples were analyzed after fortification at increasing delta9-THC concentrations covering its calibration curve from 1 to 100ng/mL and exo-THC at 1ng/mL; increasing concentrations of exo-THC in triplicate at 2ng/mL, 5ng/mL, and one sample at 20ng/mL where delta9-THC was constant at 3ng/mL; and finally, exo-THC only in duplicate at 3ng/mL.

Result: An enzyme-linked immunosorbent assay (ELISA) cross-reactivity experiment at the cut-off concentration of 10ng/mL showed exo-THC had a cross-reactivity of 52% in blood and 61% in urine. The interference data showed blood samples containing exo-THC can be distinguished from those containing delta9-THC by the retention time difference relative to the delta9-THC-d3; exo-THC eluted approximately at 6.582 minutes, before delta9-THCd3, whereas delta9-THC eluted after delta9-THC-d3. Retention times of all samples were within the acceptance criteria for delta9-THC of ±2%. If high concentrations of exo-THC starting at ≥20ng/mL were mixed with low concentrations of delta9-THC (3ng/mL), the presence of exo-THC could be identified by the relative shift of the analyte retention time. However, if delta9-THC concentration was equal to or greater than exo-THC concentration in mixed samples, the two compounds could not be distinguished as they coeluted without the noticeable change to the analyte retention time relative to the internal standard. All other cannabinoids included in the exogenous study at 10-500ng/mL did not cause misidentification as ion ratios were outside of ±20% acceptance criteria or significant overlapping peaks of >20% were present.

Discussion: Analytical challenges can arise when isomers of analytes of interest cause interference in toxicological data. It is crucial to be aware of isomers and other emerging substances that can cause hurdles in analysis and interpretation of analytical results. Based on data from an extensive interference study, the HFSC Toxicology section were able to notify stakeholders of new limitations and implement a practice of reporting positive delta9-THC results only if the analyte elutes after delta9-THC-d3 because if the compound elutes before the internal standard, this can be an indication of the possible presence of exo-THC. The laboratory is currently working on optimizing this method further to improve separation for reported analytes.

Development and Validation of a Screening and Confirmation Method of 947 Substances by LC-QTOF-MS for Routine Application in Forensic Toxicology Casework

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Introduction: The abuse of drugs has posed a substantial and longstanding social and health concern, with the more recent proliferation of various Novel Psychoactive Substances (NPS) in the illegal drug market introducing additional threats to public health and challenges to forensic toxicologists. This emergence of NPS has increased the number of substances necessary for screening in forensic toxicology laboratories, including novel opioids, novel benzodiazepines, and synthetic cannabinoids, among others, while maintaining testing of traditional medications and recreational drugs. Historical screening techniques like immunoassays and nominal mass spectrometry coupled with chromatography have demonstrated great utility within forensic toxicology, however their inability to keep pace with the constantly evolving illicit drug landscape has prompted a shift towards employing high-resolution mass spectrometry (HRMS) techniques to achieve large multi-class analysis.

Objectives: The purpose of this study was to develop, validate and implement a rapid and comprehensive quadrupole time-of-flight mass spectrometry (QTOF-MS) method for the identification and confirmation of over 900 compounds in the blood and urine of routine casework, utilizing untargeted extraction and untargeted sequential window acquisition of all theoretical mass spectra (SWATH) coupled with a targeted processing method.

Methods: All validation studies were designed and evaluated in accordance with the national ANSI/ASB Standard Practices for Method Validation in Forensic Toxicology, ANSI/ASB Standard for Mass Spectral Data Acceptance in Forensic Toxicology, and ANSI/ASB Standard for Identification Criteria in Forensic Toxicology. Samples were extracted by protein precipitation followed by size-exclusion filtration. Separation was achieved using a Kinetex 2.6 µm Biphenyl column. Eluents comprised of aqueous mobile phase A (MPA) of 10mM ammonium formate in deionized water at pH 4.5 and organic mobile phase B (MPB) consisting of methanol with 0.1% formic acid. Initial conditions of 5% MPB and 95% MPA were held until 1.0 minutes and then gradually increased to 95% MPB throughout 11.5 minutes. A combined flow rate of 1.2 mL/min was consistently maintained. Target analytes were identified via a comprehensive mass acquisition method utilizing SWATH with a mass range of 25-550 Da divided into 20 variable width windows. Fragmentation occurred using a collision energy spread of 35 +- 15 eV. Data were analyzed using vendor software with a comprehensive custom-calculation processing method built in-house. To optimize data automation and identification criteria, rigorous testing of previously acquired casework samples was conducted.

Results: Chromatographic resolution was achieved for all 947 target analytes. Target-target selectivity studies were conducted on all analytes. Additional selectivity studies were performed on 407 isomeric analytes that eluted within the same retention time window, confirming that many of these compounds could be sufficiently distinguished. Following the injection of 40 samples from authentic casework (matrix interference studies), any matrix artifacts equal to or greater than the limit of scope were noted, however none passed identification criteria and/or were separable by retention time and therefore did not interfere with identification. Carryover, ion suppression/enhancement, limit of scope (or limit of detection), processed sample stability, and applicability studies were assessed and deemed fit for purpose.

For enhanced processing, a total of 280 individual calculations were created using the coding programming available within the new features of the vendor software, and subsequently divided into 18 sections with 51 subsections for organizational purposes. A total of 21 conditional lookup tables were also built, along with custom flagging rules specific to qualitative flagging of QTOF data (i.e., mass error, retention time error, isotope pattern and library score).

Discussion: Although HRMS based technologies have been utilized in forensic toxicology testing regimes, use of the technique is relatively novel and largely limited to ad-hoc screening, with other tandem-based technologies used as confirmation. The extensive data generated by HRMS techniques constrains their routine adoption in forensic testing. Therefore,

the development, optimization and validation of this comprehensive and efficient method heavily relied on the construction of automated data analysis parameters. The use of custom calculations, flagging rules and conditional lookup functions has automated data processing systematically, and minimized transcription error, human error, and improves efficiency. Results from this described method are sufficient for use as either a screening or confirmational technique.

Automated and rapid analysis of over 200 drugs in blood and urine for DUID, DFC, and postmortem investigations

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Introduction: As driving under the influence of drug (DUID), drug-facilitated crime (DFC), and postmortem casework becomes more abundant and increasingly complex, forensic toxicology laboratories are often pushed to develop processes that are more efficient, broader in scope, and more sensitive, all while on a limited budget and keeping turnaround times low. Further, as novel psychoactive substances (NPS) come in and out of prevalence, the scope of testing must expand to accommodate these changes.

Objectives: The aim was to increase efficiency in laboratory operations by executing the following objectives: (1) develop a singular method for the detection of over 200 drugs and metabolites in blood and urine in DUID, DFC, and postmortem investigations through the consolidation of two existing analytical methods, and (2) automate the extraction of the analytical method.

Methods: The developed extraction method utilizes 0.15 mL of blood or urine, and a protein precipitation with size-exclusion filtration, followed by evaporation and reconstitution prior to analysis by LC-MS/MS. Target analytes include 226 drugs and metabolites including amphetamines, analgesics, anesthetics, anticonvulsants, antidepressants, antihistamines, antipsychotics, barbiturates, benzodiazepines, cannabinoids, cardiac drugs, cathinones, cocaine and metabolites, decongestants, dissociatives, hallucinogens, miscellaneous drugs, muscle relaxants, opioids, and Z-drugs. MRM transitions for the vast majority of target analytes were derived from two existing in-house analytical methods, with several new drugs added to the scope due to trends indicating an increase in prevalence within San Francisco. Targets were assessed qualitatively in urine. In blood, the most prevalent analytes in authentic casework (108) were assessed quantitatively, while the others were assessed qualitatively. The extraction method was developed on the Hamilton VANTAGE automated liquid handler. Batch processing was streamlined utilizing Sciex MultiQuant software in combination with an in-house developed automated query.

Results*:* The performance of the developed method was assessed by a comprehensive validation protocol, ultimately meeting ANSI/ASB Standard 036 method validation requirements. Limits of detection met sensitivity recommendations set by the U.S. National Safety Council's Alcohol, Drugs and Impairment Division's Recommendations for DUID investigations, and were suitable for other forensic casework including postmortem and DFC investigations. Accuracy and measurement of uncertainties were also appropriate, particularly important in DUID casework. The extraction method was developed on the Hamilton VANTAGE automated liquid handler, increasing efficiency and throughput within the laboratory.

Discussion: The large number of targets from a wide range of drug classes allows the laboratory to stay informed on current drug prevalence within the population, and to keep up with the ever-changing NPS landscape in real-time. Method validation identified analytes that may undergo conversion into or interfere with the identification of other target analytes. As a result, target analytes were divided into two sub-mixes in which spiked standards such as limits of detection, calibrators, and quality controls can be analyzed in the absence of the interfering analyte. The consolidation of the two analytical methods produces a much broader analytical scope while utilizing less staff time and resources in extracting and processing samples.

The extraction method was developed on the Hamilton VANTAGE automated liquid handler, mitigating human error, increasing throughput and traceability, while decreasing repetitive motion for analysts. However, in the event of instrument maintenance or downtime, the extraction method can alternatively be performed manually by the analyst, improving versatility in everyday laboratory operations.

By increasing analytical scope, a laboratory may run fewer assays and batches, saving on both time and cost. Combined with the use of automated liquid handling, this highly efficient and robust process streamlines DUID, DFC, and postmortem casework within the laboratory in a time-sensitive manner.

S30

Update: OSAC Forensic Toxicology Subcommittee and the Development of a Standard Practice and Standard Test Method for the Analysis of Volatiles

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Introduction: Over the past several years, the Forensic Toxicology Subcommittee of the Organization of Scientific Area Committees (OSAC) for Forensic Science has been drafting standards and best practices. Once completed within the OSAC, the documents are submitted to the Academy Standards Board (ASB), an ANSI-accredited Standards Development Organization (SDO), to solicit public comments and finalize the documents as American National Standards. Of recent, the Forensic Toxicology Subcommittee has been drafting a standard practice for the identification and quantitation of volatiles in biological fluids as well as a standard test method for the identification and quantitation of ethanol in blood. Although standard practice documents are not new to the forensic toxicology community, minimum requirements for the performance of analytical procedures for the identification and quantitation of specific compounds is. Further, the creation of a standard test method is new to the field of forensic toxicology and has the potential to facilitate consistency amongst laboratories performing blood ethanol analysis.

Objectives: The Forensic Toxicology Subcommittee is drafting two documents, one serving as a standard practice while the other serves as a standard test method. The standard practice document establishes the minimum requirements for the identification and quantitation of acetone, ethanol, isopropanol, and methanol in biological fluids. The creation of a minimum standard for volatiles analysis serves multiple purposes within the forensic toxicology community. Specifically, it serves to address the omission of ethanol and other volatiles from the requirements of ANSI/ASB Standard 113*, Standard for Identification Criteria in Forensic Toxicology*. The standard test method provides detailed requirements, similar to a standard operating procedure, for the analysis of ethanol in blood. The development of a standard test method has the potential to facilitate standardization across laboratories throughout the United States. This presentation will explain the differences between the standard practice (minimum standard) and standard test method including the advantages of having both as American National Standards.

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

Discussion: A update on current OSAC standard practice and standard test method pertaining to ethanol/volatile(s) analysis will be provided. This presentation will promote awareness to stakeholders on the developments of new documents within the Forensic Toxicology Subcommittee of the OSAC.

S31

The Dark Side of the SHROOM: A Retrospective Study of Psilocin Identified in Post-mortem Cases in Miami-Dade County, Florida

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Introduction: At the Miami-Dade Medical Examiner Department (MDME) the detection of traditional stimulants and hallucinogenic drugs has become more prevalent in post-mortem casework. More recently, the detection of psilocin has been reported in an array of cases, ranging from poly-drug toxicities to suicides.

The Psilocybin Mushroom (*Psilocybe ovoideocystidiata)* is a fungus colloquially known as "Magic Mushrooms" or "Shrooms". The "magic" comes from its potential to induce psychedelic effects due to psilocybin and its active metabolite psilocin. These compounds act as agonists at the 5-hydroxytryptamine $(5\text{-HT})_{2A}$ subtype receptors. When ingested, it produces psychedelic effects including a calmed mental state, euphoria, and visual and auditory hallucinations, making it difficult to differentiate between fantasy and reality. While popularly utilized in recreational settings, the benefits of psilocybin have been investigated for therapeutic uses as well as for the treatment of depression, obsessive compulsive disorder, alcoholism, and drug addiction.

Objectives: The objectives of this presentation are to present an overview of post-mortem cases at the MDME from 2018 to May 2024 in which psilocin was detected. Details such as demographics, manner of death (MOD), cause of death (COD), and overall trends will be discussed, as well as some case examples.

Methods: At the MDME, psilocin is identified either by gas chromatography paired with mass spectroscopy and nitrogen phosphorus detection (GC-NPD/MS), or by liquid chromatography paired with ion trap mass spectrometry ($LC\text{-}IonTrap\text{-}MS^n$). Using the laboratory's case management system, demographics were analyzed for all positive psilocin cases from 2018 to May 2024 including age, race, sex, as well as MOD, and COD. Psilocin was identified in blood, gastric contents, urine, brain, and liver.

Results: The table below depicts the demographics, MOD, and COD. Some cases will be selected to highlight and explore further during the presentation.

CASE	YEAR	SEX	RACE	AGE	MOD	COD
1	2018	M	W	22	Accident	Blunt Force Injuries
2	2020	F	W	27	Accident	Acute Polydrug Toxicity (Fentanyl, Acetyl Fentanyl, Alprazolam, Cocaine, Amphetamine, & MDMA)
3	2020	M	W	5	Natural	Complications of Abdominal Compartment Syndrome & Chronic Constipation
4	2020	M	B	38	Accident	Acute Combined Drug Toxicity (Fentanyl Acetyl Fentanyl, Heroin, Cocaine, Ethanol, Eutylone, & Psilocin
5	2020	F	B	21	Homicide	Gun Shot Wounds
6	2021	M	W	24	Suicide	Hanging
7	2021	F	W	20	Accident	Acute Combined Drug Toxicity (Fentanyl, Ketamine, Alprazolam, cocaine, Methamphetamine, MDMA, N-Ethyl Amphetamine, Eutylone, N,N-Dimethyltryptamine, & Psilocin)
8	2021	M	W	25	Accident	Acute Combined Drug Toxicity (Fentanyl, Fluorofentanyl, Cocaine, & Alprazolam
9	2021	F	W	27	Accident	Multiple Blunt Force Injuires
10	2022	F	W	38	Accident	Combined Toxic Effedts of Cocaine, N,N-Dimethylpentylone, Fentanyl, Fluorofentanyl, Alprazolam, & Ethanol
11	2022	M	W	18	Accident	Blunt Force Injuries
12	2022	M	W	49	Suicide	Hanging
13	2023	F	W	56	Natural	Hypertensive Cardiovascular Disease
14	2024	F	W	40	Accident	Acute Combined Drug Toxicity (Fentanyl & Acetyl Fentanyl)

Discussion: Psilocin is best identified using the LC-IonTrap-MS^n method, due to its high sensitivity. The MDME continues to identify psilocin in casework, and although it is not currently quantified, its identification can provide useful information to the medical examiner when determining cause and manner of death. Psilocin itself is not typically fatal, but its psychoactive and physiological effects can potentially be ruled as a contributory factor. Despite being unable to determine whether mushrooms were ingested for recreational or medicinal purposes, the MDME is no stranger to "traditional" drugs coming back into circulation. With the rise in the interest of exploiting its therapeutic benefits, this may lead to an increase in its identification in post-mortem case work, especially with the implementation of more sensitive instrumentation/methodologies.

Postmortem forensic toxicology interpretation: a likelihood ratio approach

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Introduction: In forensic toxicology, modern analytical techniques have enabled the identification and quantification of xenobiotics with a high accuracy. In postmortem cases, experienced forensic toxicologists will interpret these analytical results based on case circumstances (e.g., elements at the scene, autopsy results, age and health conditions of the deceased, etc.) and the existing literature (case reports, internal or external databases of cases). The final report in many cases will contain a mention such as “sub-therapeutic”, “therapeutic”, “toxic” or “fatal” accompanying the xenobiotic identification and quantification.

A number of reports and guidelines throughout the world have been highlighting the need for forensic practitioners to provide objective, transparent and sound opinions. This can be achieved, amongst others, by the use of evaluative opinions, where a likelihood ratio is assigned to the analytical results and used to communicate their probative value in the report.

Objectives: This work aims to lay the foundation for a likelihood ratio approach to postmortem forensic toxicology interpretation: the roles and responsibilities of each individual (e.g., forensic toxicologist, coroner, pathologist), how to generate a likelihood ratio (including the characteristics of the data set required for a robust result) and how to incorporate the likelihood ratio into the cause of death evaluation.

Methods: Bayesian statistics allows the introduction of new evidence (e.g., toxicology results) to update an opinion about a question at hand (e.g., cause of death). This is done through Bayes’ Theorem:

Prior Odds X Likelihood Ratio (LR) = Posterior Odds

The prior odds of cause of death, before the toxicology results are considered, are assigned by the individual ruling on the cause of death (forensic pathologist, coroner or medical examiner, depending on the jurisdiction). The LR provided by the toxicology results is assigned by the forensic toxicologist.

Broadly defined, the forensic toxicology LR would be the probability of observing the test result, given that the detected drug was the cause of death, divided by the probability of the test result, given that death was through some other cause (the drug was not the cause of death).

To evaluate an LR, the toxicologist needs to rely on a pair of datasets: one in which death was attributed to the drug being studied, and one in which the drug was detected and quantified, but death was attributable to some other cause. For each of these groups, labeled the “Death” and “Control” groups, a probability density function (PDF) is generated. The LR at a given concentration is obtained by dividing the probability in the Death group by the probability in the Control group at said concentration.

Calculations on dataset and simulations were performed in RStudio (version 2023.12.1+402 or above) using the programming language R (version 4.3.3 or above). Datasets of xenobiotics concentrations in postmortem cases were obtained from the published literature.

Results: Simulations performed show that to obtain robust LR results, both the “Death” and “Control” datasets used should contain at least 20 cases and be modelled to a known distribution (e.g., log-normal), rather than using a tool

such as Kernel density estimation.

A case example with ethanol will be detailed to illustrate the likelihood ratio approach to postmortem forensic toxicology interpretation.

Discussion: Much work remains to be done with regards to refining the Bayesian model with further forensic toxicology considerations (such as polydrug consumption, tolerance and postmortem redistribution) and explore the best approaches to communicate LR to other forensic practitioners, juries and the wider judicial system. It is important to note that the goal of this approach is not to give a better, or more accurate interpretation than the methods currently used. The goal is to provide a more transparent conclusion; as all elements contributing to the interpretation are described and quantified. This avoids potential biases such as the snowball effect; which occurs when for example both the forensic pathologist and toxicologist give weight to a particular piece of evidence (e.g., drug paraphernalia found on the scene) in orienting their decision.

Opioids in Overdose Cases in Alabama (2022-2024)

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Introduction: Since the late 1990's, the opioid epidemic has plagued America resulting in hundreds of thousands of overdose-related deaths from the use of prescription drugs (e.g. hydrocodone) or illicit opioids like heroin, and now fentanyl. An overdose occurs when someone consumes more than the typical or prescribed amount of a substance sometimes resulting in their death. Opioids are not to be confused with "opiates", although the words are used interchangeably by most people. Opiates are naturally derived from the poppy plant, while opioids are compounds that are synthesized in a laboratory. Opioids are mainly used for pain relief and at the onset of the opioid epidemic an increase in over-prescribing and misuse of opioids contributed to many deaths. Most recently, the use of fentanyl and fentanyl analogues have significantly contributed to the increase in overdose deaths across America and specifically in Alabama.

Objective: To evaluate the trends in opioids detected in casework from 2022 to 2023 in overdose cases where evidence was submitted to the toxicology section of the Alabama Department of Forensic Sciences (ADFS).

Methods: Blood specimens were screened using immunoassay technology using a Randox Evidence + Analyzer. Blood, urine, and vitreous humor specimens were confirmed using liquid-liquid or solid phase extraction followed by gas chromatography/mass spectrometry (GC/MS) or liquid chromatography-tandem mass spectrometry (LC/MS/MS) analysis. Oral fluid specimens were analyzed utilizing an in-tip Dispersive Pipette extraction (DPX) technique by a Hamilton Starlet automated system when blood specimens were presumptive positive for heroin. All methods of analysis were validated according to ANSI/ASB and SWGTOX Standards. 1,900 and 1,996 total overdose cases were evaluated in 2022 and 2023, respectively. The prevalence of each opioid in overdose case types for 2022 and 2023 in blood, unless otherwise stated, were calculated. The opioids of interest within ADFS Toxicology casework included buprenorphine, codeine, fentanyl, fentanyl analogues, heroin, hydrocodone, hydromorphone, meperidine, methadone, oxycodone, oxymorphone, morphine, and tramadol. The metabolite of heroin, 6-monoacetylmorphine (6-MAM), was used as a marker of heroin use.

Results: The total number of opioid overdose cases in 2022 and 2023 was 961 and 1,023, respectively, resulting in a 6% overall increase in opioid overdose cases at ADFS. The number of fentanyl overdoses increased from 707 in 2022 to 741 in 2023. Since the monitoring of fentanyl overdoses began in 2012, the 2023 total represents the highest reported number to date. Historical trend analysis of fentanyl analogues and designer opioids detected in casework were monitored from 2015 to 2023. Fluorofentanyl and mitragynine were the highest reported fentanyl analogue and designer opioid targets from 2022 to 2023 across overdose case types. The prevalence of mitragynine increased by 50% from 2022 to 2023. However, the prevalence of heroin and oxycodone in overdose case types decreased by 37% and 10%, respectively, from 2022 to 2023. Since the introduction of xylazine to the ADFS scope of analysis, in 2023, the number of reported cases has increased almost fourfold in the first six months of 2024. There were 352 fentanyl reported cases in the first six months of 2023 compared to 255 reported cases in the same time period of 2024.

Discussion: Fentanyl and fentanyl analogues remain an issue for Alabama as their presence continues to grow and change year by year. In 2023, fentanyl was detected in 741 overdose cases, the highest ever in a single year in Alabama. However, the presence of fentanyl analogues and designer opioids slightly decreased from 2022 to 2023. ADFS data has shown that there appears to be a consistent decline in 6-MAM in cases from 2020 to 2023, implying a decrease in heroin use. Despite small decreases in fentanyl analogues from 2022 to 2023, the opioid epidemic remains a public health concern.

Tizanidine: The Other Alpha-2 Adrenergic Agonist

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Introduction: Alpha-2 adrenergic receptor agonists are established therapeutic medications that are used for conditions such as hypertension, pain disorders, muscle relaxation, spasticity, opioid withdrawal, insomnia, and sedation. While forensic toxicologists may be familiar with more common alpha receptor agonists, tizanidine is a less frequently encountered drug for which minimal postmortem data exists. Tizanidine therapeutic levels found in plasma are reported in the range of 0.0025-0.015 mg/L, although CYP1A2 inhibitors such as ciprofloxacin or fluvoxamine can greatly increase tizanidine levels and thus potentially toxic effects. Due to its high potency, tizanidine may be easily missed by postmortem laboratories that are not targeting this drug or carefully inspecting untargeted data for its presence.

Objectives: This presentation will share instructional information on tizanidine as well as case studies and aggregate data from postmortem cases involving tizanidine at the North Carolina Office of the Chief Medical Examiner (NC OCME). These data will demonstrate a qualitative chromatogram comparison of non-toxic and lethal levels of tizanidine, concentration ranges observed, and case circumstances in which tizanidine appears causative or additive to death.

Methods: Specimens were screened for common and novel drugs via a targeted LC/MS method which did not include tizanidine, an untargeted GC/MS organic base method, and/or outside reference laboratory; presumptive positive tizanidine findings were confirmed and quantitated via outside laboratory. Case details were analyzed to determine trends and numerical ranges for suicidal, accidental, and incidental tizanidine-positive cases.

Results: NC OCME aggregated quantified data for 13 postmortem tizanidine cases from 2018-2024. Seven of the cases were clearly documented medication overdose suicides, four of which included a note referencing tizanidine consumption or an empty tizanidine bottle. Two cases were incidental for tizanidine (a natural death and a homicide by gun), three cases were ruled accidents, and one has cause and manner of death ruling still pending. Quantified amounts for the suicides ranged from 0.002 mg/L (in an antemortem sample drawn 4.5 hours after hospital admission) to 38 mg/L. The case with 0.002 mg/L of tizanidine had a suicide note referencing an intentional overdose of tizanidine, but was additionally positive for duloxetine, metoprolol, pregabalin, and trazodone. Tizanidine concentrations for the incidental cases were 0.048 mg/L and 0.050 mg/L, and the accidental cases ranged from 0.17 mg/L to 1.5 mg/L.

Suicide cases with tizanidine values at the higher end of the range (38 mg/L in femoral blood and 8.6 mg/L in iliac blood) were also positive for extremely high levels of another substance (>10 mg/L oxycodone in femoral blood and >1000 mg/L acetaminophen in iliac blood, respectively). A suicide case with a concentration of 1.0 mg/L of tizanidine in femoral blood was found in a decedent that additionally had a plastic bag covering her mouth and nose. Three suicide cases with other additive substances included tizanidine concentrations of 0.34 mg/L, 0.71 mg/L, and 0.83 mg/L.

Discussion: While alpha-2 adrenergic agonists were developed as therapeutic drugs, their relevance within the world of illicit use has recently resulted in focused attention. Clonidine is used as a therapeutic agent to alleviate opioid withdrawal symptoms, and xylazine and medetomidine have appeared as cutting agents/adulterants within the illicit opioid supply. Thus far tizanidine is not following this pattern.

Interest in tizanidine at the NC OCME was sparked by its potency, infrequency, and the relative lack of postmortem data available in the literature. Depending on laboratory workflow, it may be beneficial for forensic laboratories to add the drug to routine or expanded targeted screening assays. Additionally, lethal concentrations may result in seemingly insignificant peaks on traditional GC untargeted basic drug extractions, so quantifying tizanidine should be considered if it is encountered depending on the details of the case and availability of funding if the test requires outsourcing.

A 5 Year Review of Postmortem NPS Trends in Travis County, Texas

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Introduction: Novel psychoactive substances (NPS) have not waned in popularity over the years despite active government regulation. New compounds with unknown toxicities are continually emerging and evolving, resulting in adverse events. These "legal highs" pose problems for toxicologists who must identify an unending variety of new drugs of abuse and continually adjust their scopes of testing. Furthermore, even though new substances are constantly emerging, only a fraction of emerging NPS gain popularity making it cumbersome for laboratories to stay abreast with current trends.

Objective: A retrospective analysis was conducted to characterize trends observed in NPS-positive postmortem cases from Travis County, Texas and surrounding counties over the past five years (2019-2023). Interesting toxicology findings from select NPS postmortem cases will also be highlighted.

Method: Postmortem specimens were collected at autopsy at the Travis County Medical Examiner (TCME) in Austin, TX. Specimens were screened for volatiles utilizing headspace dual column gas-chromatography (GC) with flame ionization detection and drugs of abuse by immunoassay (ELISA). Qualitative drug screens were performed with either acid/neutral and alkaline GC-mass spectrometry (MS) following liquid-liquid extraction or via liquid chromatography quadrupole time-of-flight mass spectrometry (LC-QTOF-MS) for the identification of 350+ drugs following protein precipitation. NPS confirmation and/or quantification involved targeted liquid chromatography-tandem mass spectrometry (LC-MS/MS), LC-QTOF-MS, and/or non-targeted full-scan GC-MS methods following various sample preparation techniques.

Postmortem cases submitted to TCME for toxicology testing between January 1, 2019 and December 31, 2023, from Travis County, TX, and surrounding counties, were queried to identify the total number of NPS-positive cases during this timeframe. Demographic (sex, race, age) and toxicological information was gathered for all NPS-positive fatalities and evaluated annually and in totality.

Results: Of 11,526 toxicological cases analyzed over the past five years, 3.7% of cases (*n*=430) tested positive for one or more NPS, with a total of 527 NPS identifications (1.2 NPS/case). NPS postmortem cases were mainly White males (80%) in their early-30's. Forty-four different NPS compounds were identified from 2019-2023 with a relatively even distribution amongst the NPS subclasses; 10 NPS accounted for 85% of all NPS identifications made. NPS benzodiazepines were the most common NPS subclass detected (249 cases; 58%), followed by NPS opioids (124 cases; 29%), synthetic cannabinoids (66 cases; 15%), and NPS stimulants & hallucinogens (15 cases; 3.5%). Variable trends were observed between the different NPS subclasses year-to-year; NPS benzodiazepine trends were heavily influenced by drug scheduling as highlighted by the observed shifts from etizolam, flualprazolam, and clonazolam towards bromazolam over the study period. Cases involving NPS benzodiazepines generally increased over time, with bromazolam accounting for the most NPS identifications (*n*=112; 21%), followed by etizolam (n=51; 9.7%), and clonazolam (identified by 8-aminoclonazolam) (n=45; 8.5%). Synthetic cannabinoids were predominantly detected from 2019-2021 with 5F-MDMB-PICA (*n*=43; 8.2%) and MDMB-4en-PINACA (*n*=22; 4.2%) being the most prevalent. NPS opioids were only frequently detected after the inclusion of fluorofentanyl (*n*=93; 18%) in 2021 and after a carfentanil spike in 2020 (*n*=21; 4.0%). Most NPS were rarely detected without other substances; however, early synthetic cannabinoids were often detected alone, or in combination with ethanol and cannabis. In 2023, approximately 80% of NPS benzodiazepine cases were detected with fentanyl and/or fluorofentanyl. Interestingly, a few NPS have re-emerged years after their initial detection, including carfentanil, a-PVP, 5F-ADB, and acetyl fentanyl.

Conclusion/Discussion: Retrospective review of the last five years of postmortem cases around Travis County, TX highlighted a low, but consistent, presence of NPS-positive cases that have continued to transform and evolve over

time. Particularly alarming, is the increased presence of NPS benzodiazepines with fentanyl in recent years. Disseminating NPS trends is imperative to reduce their public safety threat and a way to ease the burden on laboratories by helping establish regional NPS testing scopes.

A "Tail" of Two Cities – When Old Synthetic Cannabinoids Become New Again

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Introduction: Synthetic cannabinoids have plagued forensic toxicologists and analytical laboratories for many years, in large part due to challenging chemistry and complex drug market trends. Synthetic cannabinoids first emerged in the late 2000s and by the mid-2010s had become the largest class of NPS, contributing to adverse events and deaths worldwide. Changes in synthetic cannabinoid markets had largely been driven by national and/or international control and scheduling efforts, with a general trend toward increasing potency. However, the global synthetic cannabinoid market changed significantly in the early 2020s following a Chinese class-wide legislative action. This led to increased diversity of chemical structures, innovations in manufacturing and distribution, and increased variability in product potency with generally less potent (or inactive) drugs appearing. As a result, today's synthetic cannabinoid landscape is even more complex, but adverse events related to synthetic cannabinoid exposures remain.

Objectives: This presentation aims to provide an updated glimpse into the synthetic cannabinoid landscape in the United States by explaining recent market changes and examining new forensic toxicology cases that contain these drugs and other substances.

Methods: Through collaborations with medical examiner/coroner offices, crime laboratories, and correctional institutions, the Center for Forensic Science Research and Education (CFSRE) receives drug materials and toxicology specimens for comprehensive testing of synthetic cannabinoids and other relevant drugs. Drug materials commonly consist of legal papers, personal correspondence, or greeting cards soaked with synthetic cannabinoid solutions, as well as plant materials and powders. Toxicology specimens consist of postmortem blood, urine, antemortem serum/plasma, and other biological fluids. Comprehensive qualitative toxicological drug testing is performed by liquid chromatography quadrupole time-of-flight mass spectrometry (LC-QTOF-MS) using a SCIEX X500R and a TripleTOF 5600+. Samples are prepared by an acidic liquid-liquid extraction to simultaneously capture synthetic cannabinoid parent drugs, metabolites, and other species. An extensive in-house database of more than 1,100 substances is used for targeted processing of acquired datafiles. When possible, quantitation of synthetic cannabinoids in blood is performed by liquid chromatography tandem quadrupole mass spectrometry (LC-QQQ-MS) using validated methods.

Results: Between 2018 and 2021, 5F-ADB (5F-MDMB-PINACA) followed by 5F-MDMB-PICA and MDMB-4en-PINACA were the most commonly encountered synthetic cannabinoids on a rolling basis. Following the Chinese ban in mid-2021, positivity dipped as previously prevalent synthetic cannabinoids disappeared from markets; however, there appears to be a rebound occurring in 2024. MDMB-4en-PINACA remains the most widely detected synthetic cannabinoid despite its DEA Schedule I status and inclusion in the Chinese class-wide ban. MDMB-4en-PINACA is now often detected alongside its precursor, MDMB-INACA, indicating changes in synthesis and manufacture. This precursor and others (e.g., ADB-INACA) have been offered for sale on gray markets with the "tail" portion of the molecule (e.g., 4en-pentyl, butyl, 5F-pentyl) being added subsequently. MDMB-BINACA (MDMB-BUTINACA) was detected in a blood sample collected after a jail death for the first time in the United States in October 2023. MMB-4en-PINACA was detected on a paper confiscated from a jail for the first time worldwide in February 2024. As of May 2024, 5F-ADB has been identified in at least five jail death cases, marking the return of this synthetic cannabinoid after years of dormancy. Interestingly, MDMB-4en-PINACA, MDMB-BINACA, MMB-4en-PINACA, and 5F-ADB can all be produced via the same common MDMB-INACA precursor.

Discussion: Synthetic cannabinoids remain of high public health concern due to involvements in medicolegal death investigations, especially among jail and prison populations. Toxicology testing for synthetic cannabinoids and metabolites should be pursued, especially using methodologies and workflows with dynamically updated testing scopes. The synthetic cannabinoid landscape continues to change in the United States and the current market is observing the re-emergence of previously prevalent drugs with a "tail-less" (precursor) commonality.

Trends in Tranq: Prevalence of Xylazine in Oral Fluid Toxicology in Michigan, Ohio and Indiana

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Introduction: Xylazine (which goes by the street name "Tranq") is a veterinary animal tranquilizer that is commonly used in combination with illicit fentanyl. Increased testing and surveillance are the first steps toward curbing abuse. Oral fluid presents an ideal matrix to capture this data due to its non-invasive and simple means of collection. We examined xylazine patterns of use and spread across 13 months of oral fluid toxicology data beginning in March 2023 for Michigan, Ohio, and Indiana.

Objectives: Our objective was to investigate trends in xylazine use and spread in Michigan, Ohio and Indiana.

Methods: Oral fluid specimens were analyzed using a combination of immunoassay screens and CLIA validated LC–MS-MS confirmation methods at Forensic Fluids Laboratories. We focused on the population of recreational drug users; samples that screened positive for at least one of the following drugs of abuse between March 2023 and March 2024 were used as the basis for measuring xylazine prevalence: methamphetamine, cocaine, opiates, and fentanyl. Xylazine ELISA was conducted based on customer request and this data was also incorporated where available. The pool of analyzed samples was largely composed of samples from child protective services and other state and county-level social services departments, with smaller contributions from probation departments, substance use disorder clinics and other health centers. LC–MS-MS confirmation data from these samples were subsequently pulled and tabulated for the following drugs: amphetamine, methamphetamine, MDMA, cocaine, fentanyl, 6-monoacetylmorphine, morphine, codeine, hydrocodone, oxycodone, tramadol, methadone, buprenorphine, dextromethorphan, PCP, delta-9 THC, and xylazine. Plots and maps were generated using NumPy, Pandas, Matplotlib and GeoPandas libraries in Python.

Results: On average over the entire span, xylazine was found in 3.41% of the total 55,691 analyzed samples. Using the three-month xylazine positivity rate, prevalence peaked at 3.76% in June 2023 and hit its low in September 2023 at 3.14%. Of the xylazine positive samples, fentanyl was present 96.5% of the time. In addition, 86.2% of xylazine samples indicated some degree of polydrug use where at least two other drug classes were present. Of note, stimulants and fentanyl were present in 61.4% of xylazine positives. Xylazine increased in prevalence among fentanyl positive samples. Using the three-month average, xylazine was found in 21.70% of fentanyl positive samples in May 2023, and was found in 30.92% in March 2024. Across the states Michigan, Ohio, and Indiana, xylazine use spread to new counties by the conclusion of our study window. The prevalence of xylazine usage varied depending on the county; some observed an increase in use, while others saw a decrease.

Discussion: The data presented represents a sampling of xylazine drug trends focusing on Michigan, Ohio and Indiana. Overall, our data lends support to the notion that xylazine is actively penetrating the illicit fentanyl market. Early on, xylazine was found in about one-fifth of fentanyl-positive samples; as of March 2024, it is approaching one-third. Based on our findings, xylazine users were most commonly polydrug users. The evolution of the "speedball" drug combination (stimulant and narcotic) now commonly includes xylazine, presenting an additional challenge for treatment providers. In Michigan, Ohio, and Indiana, the overall xylazine prevalence decreased between May 2023 and March 2024 (2.82%, 4.54% and 4.15%, and 2.75%, 4.40%, and 3.41%, for each state and time respectively). However, county-level xylazine prevalence displayed dynamic trends in use over this period, with some counties worsening while others improving overall. With these findings, we aim to promote collaboration among local, state and federal agencies to develop strategies to curb the use and spread of xylazine.

Characterization of Reactive Metabolites for Eight NPS Using Glutathione and N-Acetylcysteine Trapping Assays

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Introduction: Through comprehensive metabolic investigations, forensic toxicologists can enhance their understanding of NPS, thereby enhancing the compendium of literature on NPS to accurately identify and interpret toxicological findings. These advancements contribute to more effective strategies for drug detection and reporting. Traditional toxicology methods screen for stable metabolites to identify potential drug exposure. However, a concerning alternative metabolic event involves the formation of reactive metabolic species, which can form irreversible covalent bonds with enzymes or other proteins. This binding can inactivate proteins, leading to immediate toxicity or long-term organ damage. Reactive metabolites have the potential to bind to macromolecular structures and are short-lived species. Detoxification of reactive metabolites requires liver enzyme trapping agents like glutathione-S-transferases (GST), which conjugate glutathione (GSH) to the metabolite. Similarly, due to its thiol group, N-acetyl cysteine (NAC) makes for another effective reactive drug metabolite trapping agent. GSH and NAC behave as nucleophiles that react with electrophilic intermediates in drug metabolism, thus allowing for their trapping behavior. Elucidating the structural changes of a parent NPS to form reactive drug species can give insight on intermediates that ultimately become stable metabolites and allow for a better biotransformation profile of NPS, that are otherwise lacking in information.

Objectives: Identify reactive metabolites of each JWH-018, 5F-APINAC, Eutylone, 4'-Methyl-α-pyrrolidinohexiophenone (MPHP), 25H-NB4OMe, 25T7-NBOMe, Despropionyl para-fluorofentanyl (DPFF), and para-Chloroisobutyryl fentanyl (pCIBF) to allow for structural elucidation of these intermediates and assess the feasibility of utilizing GSH and NAC trapping assays to determine NPS reactivity and pathway of forming stable adducts.

Methods: A 25 mM ammonium bicarbonate buffer, acidified to pH 7.4 with formic acid, was prepared. An aliquot of individual NPS standards was dried in a vacufuge at 45°C for 30 minutes to remove methanol. Separately, cofactors (1 mM NADPH, 1.5 mM glucose-6-phosphate (G6P), 1.5 mM MgCl2 were prepared. The dried NPS was reconstituted in the buffer, then combined with human liver microsomes (HLM) (0.5 mg/mL) and glucose-6-phosphate dehydrogenase (G6PD) (0.2 U/mL) for a final volume of 100 µL and 500 micromolar NPS concentration including 1 mM GSH or NAC. The mixture was incubated at 37°C with constant mixing (120 rpm) for 4 hours, then centrifuged at 10,000 g for 15 minutes. A 200 µL supernatant aliquot was analyzed by LC-QToF-MS for metabolite identification.

LC-QToF-MS analysis was conducted using an Agilent 1290 Infinity HPLC and Agilent 6546 QToF-MS with a Zorbax Eclipse Plus C18 column. A mobile phase of 0.1% formic acid in water (A) and acetonitrile (B) was used, with a gradient flow of 0.500 mL/min from 5% to 95% B over 15 minutes. Scans were conducted in positive and negative mode. Data processing was completed using Agilent MassHunter Qualitative Analysis 11.

Results: The metabolic assays conducted in this study facilitated the screening and confirmation of the eight parent structures in each in vitro assay. At least two trapped reactive metabolite species were detected for each of the eight NPS, with DPFF incubated with GSH showing the highest number of trapped reactive metabolites. Both GSH and NAC trapped metabolites, with conjugation occurring primarily at saturated ring structures.

Discussion: Several GSH and NAC conjugated metabolites were identified from the eight NPS in this study. The identification of these conjugates indicates that reactive metabolites were formed and subsequently neutralized by conjugation with GSH and NAC. This conjugation process often suggests that the reactive metabolites underwent initial hydroxylation through an epoxidation mechanism. The results of this study demonstrate that GSH and NAC can be effective trapping agents due to their strong nucleophilic properties, which allow them to readily conjugate reactive metabolites, thereby allowing structural identification of reactive metabolites and overall improvement of metabolic profiles for NPS.

Novel Psychoactive Substances: Comparative Performance of LC-QTOF-MS Acquisition Methods for Nontargeted Analysis in Urine and Whole Blood

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Introduction: The landscape of psychoactive substances is constantly evolving, with novel compounds emerging at an unprecedented rate. Traditional analytical methods struggle to keep pace with this influx, often failing to detect or identify these new substances. Nontargeted analysis has emerged as a crucial tool in this context, offering a comprehensive approach that can uncover both previously known and unknown compounds. High-resolution mass spectrometry (HRMS), particularly liquid chromatography coupled with quadrupole time-of-flight mass spectrometry (LC-QTOF-MS), has emerged as a cornerstone technology in this endeavor, enabling comprehensive screening and providing detailed molecular information with exceptional accuracy and resolution of the compounds without prior knowledge. As the forefront of analytical innovation, nontargeted analysis holds immense promise for addressing the challenges posed by emerging psychoactive substances, offering insights that can inform public health interventions and regulatory measures. Within this framework, the selection of MS data acquisition methods, such as Auto MS/MS and All Ions fragmentation becomes pivotal, shaping the effectiveness and reliability of the analytical process.

Objectives: This study aims to elucidate the efficacy of LC-QTOF-MS in nontargeted analysis for detecting and characterizing new psychoactive substances (NPS) in urine and whole blood samples. By comparing two acquisition methods, Auto MS/MS and All Ions MS/MS, our objective is to discern their respective abilities to identify known and unknown NPS qualitatively with precision and sensitivity.

Methods: Drug-free human urine and whole blood obtained from commercial sources were spiked with a 40-compound NPS test mixture representing various chemical classes including isomers, metabolites, and closely eluting analytes. Dilute-and-shoot and crash-and-shoot extraction methods were used for urine and whole blood samples, respectively. The samples were subjected to nontargeted analysis using an Agilent 6546 LC-QTOF-MS and Agilent MassHunter Auto MS/MS and All Ions MS/MS acquisition methods under optimized conditions in positive ESI ionization mode at specific collision energies of 10, 20 and 40 eV. Data processing and compound identification were performed using various software tools, including Agilent MassHunter Qualitative and Quantitative, Agilent Suremass for high-resolution MS profile data processing, Sirius (FSU Jena) for fragmentation data, a custom built in-house PCDL, and an online database for spectral library searching like HighResNPS and Forensic Tox EPA (*Environmental Protection Agency*). A scoring system was adapted to evaluate the comparison of selected figures of merits including sensitivity, selectivity, linearity, precision, and matrix effects for each method.

Results: Our findings reveal distinct differences between the Auto MS/MS and All Ions fragmentation acquisition methods in terms of their ability to identify multiple NPS in a single test mixture. High resolution data with mass accuracy <2 ppm was achieved for both methods. Calibration curves showed excellent linearity, with R^2 values ranging from 0.990 to 0.999, and the precision across all compounds was under 10% CV. Auto MS/MS exhibited superior sensitivity and selectivity for targeted analytes, while All Ions fragmentation provide broader coverage and enhanced detection of unknown compounds. Furthermore, All Ions fragmentation demonstrated greater resilience to matrix effects and interference, leading to more reliable identification of NPS across diverse sample types.

Conclusion/Discussion: Our study highlights the critical importance of acquisition method selection in LC-QTOF-MS-based nontargeted analysis of NPS in biological matrices. While both Auto MS/MS and All Ions fragmentation offer unique advantages, their performance characteristics must be carefully considered in the context of specific analytical goals and sample complexities. The comprehensive evaluation provided herein serves as a valuable resource for researchers and forensic practitioners seeking to optimize their methodologies for NPS detection and identification.

Pharmacological characterization and forensic case series of emerging 2-benzylbenzimidazole 'nitazene' opioids

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Introduction: In response to class-wide bans on fentanyl analogues, 2-benzylbenzimidazole 'nitazene' opioids have recently emerged on recreational drug markets worldwide. With potencies exceeding that of morphine and even fentanyl, nitazene use is particularly dangerous. Although numerous nitazenes have been extensively examined, only few reports have conducted a systematic comparison of the effect of different structural modifications to the 2-benzylbenzimidazole core structure on μ-opioid receptor (MOR) activity. Furthermore, the constant emergence of new analogues, combined with increased detection and intoxication rates, signals a potentially looming nitazene crisis that requires close monitoring.

Objectives: The *in vitro* MOR activation potential of 9 previously uncharacterized nitazenes, along with their close analogues was investigated. Specifically, we focused on MOR activation by 'ring' nitazenes (i.e., *N*-pyrrolidino and *N*-piperidinyl analogues), *N*-desethyl nitazenes, and 'des'-nitazenes (lacking the 5-nitro group). In addition, bridging the pharmacological data with case data, this study reports on the identification and toxicological analysis of etodesnitazene, *N*-desethyl etonitazene, *N*-desethyl isotonitazene, *N*-pyrrolidino metonitazene, and *N*-pyrrolidino protonitazene in 85 forensic cases from the U.S., Canada, and the United Kingdom.

Methods: Two complementary *in vitro* MOR activation assays were employed to evaluate the MOR activation potential of a set of 25 differentially substituted 2-benzylbenzimidazole opioids: a NanoBiT®-based β-arrestin 2 recruitment assay and a GloSensor® cyclic adenosine monophosphate (cAMP) assay. An LC-(Q)TOF-MS-based forensic toxicology drug screening, followed by standard addition LC-MS/MS-based quantitation of etodesnitazene, *N*-desethyl isotonitazene, *N*-pyrrolidino metonitazene, and *N*-pyrrolidino protonitazene in authentic casework samples was conducted by the Center for Forensic Science Research and Education (CFSRE, Philadelphia, USA).

Results: The obtained potency and efficacy values allow to build further on previously established structure-activity relationships for nitazenes. Our results show that 'ring' modifications overall yield highly active compounds, with *N*-pyrrolidino substitutions generally being more favorable for MOR activation than *N*-piperidine substitutions. Furthermore, the importance of the nitro group at the 5-position of the benzimidazole ring was demonstrated, as its removal consistently led to a 10- to 100-fold reduction in potency. The *N*-desethyl modification is overall well-tolerated in terms of MOR activation potential, and generally lowers the potency ~6- to 14-fold. Intriguingly, *N*-desethyl isotonitazene showed an opposite trend, and was ~3 times more potent than isotonitazene. Consistent with previous findings for other nitazenes and in line with what could be anticipated from the *in vitro* pharmacological characterization, blood concentrations obtained for etodesnitazene, *N*-desethyl isotonitazene, *N*-pyrrolidino metonitazene, and *N*-pyrrolidino protonitazene in the evaluated forensic case series (n = 85) were generally in the low ng/mL range. Notably, with the exception of one case, all of the evaluated cases were polydrug intoxications, with nitazenes being most commonly found in combination with fentanyl (56% of cases). Furthermore, in the instances where *N*-pyrrolidino metonitazene was detected, it was predominantly found in combination with *N*-pyrrolidino protonitazene.

Discussion/Conclusion: This study describes the largest case series of intoxications involving nitazenes to date and is the first to report on the identification and toxicological analysis of etodesnitazene, *N*-desethyl etonitazene, *N*-desethyl isotonitazene, *N*-pyrrolidino metonitazene, and *N*-pyrrolidino protonitazene in forensic toxicology casework from North America. The case data combined with the comparative pharmacological evaluation of a diverse panel of nitazenes carried out in this study may contribute to raising awareness, as well as strengthen preparedness and harm reduction efforts.

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The History of Breath Alcohol Detection

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Introduction: In the 19th century, law enforcement officials dealt with the problem of alcohol abusers by imprisoning them until they were sober. In the 20th century, the advent of high-speed transportation combined with alcohol use, created a need for alcohol testing. Automobiles traveling at over 100 feet per second on the freeway are unforgiving of drivers who are impaired by alcohol use. The same is true for pilots of aircraft, ships or operators of heavy equipment. People who operate complex equipment with their judgment impaired by alcohol may not only be a danger to themselves, but impact the safety of others.

Until recently, the main application of alcohol testing was to traffic law enforcement. The intent was to identify people suspected of driving under the influence of alcohol and remove them from the road. After arrest, law enforcement officers gave the subject a chemical test to determine their blood alcohol concentration. Subjects were either released or incarcerated and prosecuted, depending on what alcohol levels were determined to be illegal as dictated by state law or jurisdictional rules. Until the mid-1940's, the primary means of measuring blood alcohol levels involved either blood or urine sample testing, both of which were time-consuming and expensive procedures. In the late 1940's, alcohol breath testing replaced blood and urine sample testing as a primary means of screening subjects and producing evidentiary results for prosecution.

Objectives: As the grandson of Dr. Glenn C Forrester, the CEO of Intoximeters Inc., a St. Louis based Corporation and the oldest breath alcohol test instrument manufacturer in the world, I have a unique perspective on how the breath alcohol instrument industry evolved. I wish to share is with the attendees.

Methods: We will discuss the evolution of measuring alcohol in the breath and we will discuss some of the varied methods of detection.

- Visual Detection of Colorimetric Change
- Photoelectronic measurements of Colorometric Change and the preserved sample
- Gas Chromatography and the preserved sample
- Infrared analysis (Single Wavelength and Multi-Wavelength analysis)
- Semi-Conductor Analysis
- Electrochemical (Fuel Cell) analysis
- Dual Sensor Analysis (Fuel Cell and Infrared)

Discussion: What is in the Future for Breath Alcohol Testing

We will conclude the presentation with a discussion of what technologies might be used in the future.

Rate of Positive Drug Results Following the ASB's Minimum Testing Standards in Postmortem and Impaired Driver Cases

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Introduction: In 2021, the AAFS Standards Board (ASB) published two documents, ASB 119 and ASB 120, which set minimum standards for testing in postmortem cases (PM) and impaired drivers (ID), respectively. The Toxicology section of the Montgomery County Coroner's Office in Dayton, Ohio developed a targeted LC/MS/MS method to screen for the recommended drugs in both PM and ID casework at or below the recommended cutoffs. The results of testing in 2023 are discussed below; 2024 data will be added before the presentation. Targeted drugs that were not recommended will also be discussed.

Objectives: To show how often the drugs recommended in the minimum standards were present in casework. The results may encourage laboratories to adopt the minimum standards for testing recommended by ASB. Conversely, a laboratory may determine that its current testing would not benefit from adopting the standards.

Methods: In 2023 a solid-phase extraction method with analysis by LC/MS/MS was performed on all PM and ID cases, when sample volume was adequate (0.5 mL). All positive results from the targeted analysis were extracted from the laboratory's LIMS system and analyzed in Excel. PM and ID casework were reported separately to not skew results between the ASB documents. Data from 2024 will be added before presentation at SOFT. Due to the analytical method being used as a traditional "screen" methodology, drugs with listed screening targets in the ASB documents were included in the analysis, along with several other drugs based on regional importance.

Results: There were a total of 2,529 PM cases analyzed in 2023. All PM cases were screened using ASB 119 – Table 1 (suspected toxicological cause of death), except for acetaminophen and salicylates. Eight drugs had positivity rates above 5%: fentanyl (30%), ethanol (23%), carboxy-delta-9-THC (20%), methamphetamine (19%), amphetamine (16%), benzoylecgonine (14%), gabapentin (9%), and diphenhydramine (6%). The following drugs were not found in any casework: pentobarbital, secobarbital, phencyclidine (PCP), carisoprodol/meprobamate, and MDMA/MDA. Several drugs not specified as targets showed positivity rates above 1%: carboxy-delta-8-THC, acetylfentanyl, fluorofentanyl, mitragynine, and xylazine.

There were a total of 542 ID cases analyzed in 2023. For ID cases, ASB recommended 24 different targets. Only six drugs had a positivity rate of more than 5%: ethanol (23%), carboxy-delta-9-THC (16%), fentanyl (9%), benzoylecgonine (6%), methamphetamine (5%), and amphetamine (5%). Four targets were not seen in any casework: MDMA/MDA, carisoprodol, and codeine. All other target drugs had positivity rates of less than 5%. Four drugs not recommended had positivity rates above 1%: 7-aminoclonazepam, fluorofentanyl, gabapentin, and xylazine.

Discussion: For laboratories considering following the ASB-published minimum standards for testing in PM or ID cases, it is important to consider the benefit in expanding their current testing strategy. In this laboratory's experience, the inclusion of the recommended targets did not greatly increase the rate of positivity for either PM or ID cases. It was noted that targeting 7-aminoclonazepam in PM cases increased the detection rate for cases in which clonazepam had been consumed by the decedent over sevenfold. The addition of drugs commonly seen in the region (e.g., fluorofentanyl, xylazine) should also be done so that local trends are not ignored.

It is also important for a laboratory to consider what is the most efficient way to analyze PM cases. Labs may choose a testing scheme based on history or create one that meets both ASB documents. For labs that perform both PM and ID casework, the decision to set one decision point for targeted drugs in both types of cases should also be considered to increase efficiency while exceeding the published guidelines. Finally, we recommend that the ASB conduct a large-scale survey to determine if changes should be made for future versions.

Analysis of 28 Phytocannabinoids and Semi-Synthetic Cannabinoids in Blood and Urine by LC-MS/MS

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Introduction: Cannabinoid products have become more widely available since the legalization of hemp in the Agriculture Improvement Act of 2018. These products, advertised to contain tetrahydrocannabinol (THC) isomers and analogs are available in various shops, including online stores. They provide users with unregulated alternatives to Δ9-THC, which is the natural psychoactive component of cannabis. Since products could contain Δ8-, Δ9-, and Δ10-THC as well as other structurally related compounds such as Δ8-iso-THC, Δ4(8)-iso-THC, and Δ9,11-THC (exo-THC), an updated analytical method was needed to differentiate these isomers to ensure accurate reporting. Ideally, this method would also address the rise of similarly related compounds such as THCV, THCB, THCH, THCP, and THC-C8, along with their hydrogenated counterparts of hexahydrocannabinol (HHC)

Objectives: This presentation will describe the development of an optimized extraction and selective LC-MS/MS confirmation method to qualitatively identify the presence of 28 cannabinoids and metabolites in blood and urine, eight of which will be quantified in blood.

Methods: Solid phase, supported-liquid, and liquid-liquid extractions were compared to identify the optimal technique for extracting polar and nonpolar cannabinoid analytes. Enzymatic and base hydrolysis methods were also evaluated to determine the optimal hydrolysis procedure for urine specimens. Thirteen LC columns and five different aqueous/organic mobile phase combinations were investigated to properly resolve critical sets of isomers. Other LC parameters evaluated included gradient, column temperature, and LC flow rate.

Limit of Detection 1 ng/mL		
Δ^8-THC*	Δ^8-THCV	(S)-HHCP
11-OH-Δ^8-THC*	Δ^9-THCV	Δ^8-THCP
Δ^9-THC*	Δ^8-THCB	Δ^9-THCP
11-OH-Δ^9-THC*	Δ^9-THCB	Δ^8-THC-C8
9(R)-HHC*	Δ^8-THCH (urine only)	Δ^9-THC-C8
9(S)-HHC*	Δ^9-THCH	
exo-THC	(R)-HHCP	
Limit of Detection 3 ng/mL		
Δ^{10}-THC	$\Delta^{6a,10a}$-THC	
Limit of Detection 5 ng/mL		
Δ^8-THCCOOH*	9(R)-HHCCOOH	Δ^8-THCVCOOH
Δ^9-THCCOOH*	9(S) HHCCOOH	Δ^9-THCVCOOH
Δ^{10}-THCCOOH		

*Quantitative. Limit of detection is also lower limit of quantitation (LLOQ).

Results: A liquid-liquid extraction was developed for blood and urine with a dual enzymatic-base hydrolysis for urine specimens. Detection and separation were achieved using a Shimadzu Nexera X3 UPLC with an Agilent Poroshell 120 PFP column (4.6 mm x 100 mm, 2.7 µm) and a Sciex 4500 QTRAP MS/MS. All analytes were resolved within 14 minutes using mobile phases of 0.1% formic acid in water and 0.05% formic acid in methanol. Additionally, Δ8-iso-THC and Δ4(8)-iso-THC were baseline separated from analytes of interest. Low limits of detection were

achieved for all analytes in blood and urine, except for Δ8-THCH due to an endogenous interference in blood. Validation results consistent with ANSI/ASB 036: Standard Practices for Method Validation in Forensic Toxicology will be presented.

Discussion: This expansive LC-MS/MS method can be used to confirm the presence of common THC isomers, such as Δ8-THC, as well as newer analogs that have not been as extensively studied, such as HHC and THCP. While THC isomers and analogs are advertised in numerous products, it is unclear if these analytes are present or are being used as a marketing ploy. Using this procedure, relevant types of phytocannabinoids and semi-synthetic cannabinoids along with their metabolites are chromatographically separated from each other and interfering synthesis byproducts. This method will be used to analyze authentic specimens and provide valuable information for laboratories seeking to accurately identify modern variations of cannabinoids in routine casework.

Comparison of Ketone Levels Using a Commercial Ketone Monitoring System to Screen for Beta-hydroxybutyrate Prior to GC-MS Analysis.

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Introduction: Ketoacidosis is a life-threatening condition that can affect people with diabetes. The condition can also occur in alcoholism, starvation, and hypothermia.***[i]*** Ketoacidosis occurs when the body starts breaking down fat at a rate that is too fast, inducing lipolysis. To determine ketoacidosis as a cause of death, ketones, specifically beta-hydroxybutyrate (BHB) must be detected and quantified. BHB is the best ketone to quantify because it contributes the most to the lowering of pH, is not susceptible to postmortem synthesis or degradation, and is only formed endogenously.***[ii]*** BHB concentrations greater than 2.5 mmol/L have been established as pathologically significant.***[iii]*** Ketone meters are point-of-care (POC) devices that offer a quick and inexpensive way to quantify ketones.***[iv]***

Objectives: This research aims to compare BHB concentrations measured at the morgue to those subsequently measured at the toxicology laboratory in Jefferson County Coroner/Medical Examiner (JCCMEO) cases from September 2022 to April 2024.

Methods: Data was obtained from the JCCMEO case database management system by performing a search for "ketone meter." Toxicology and Medical Examiner (ME) data in each case were exported, de-identified and sorted. Paired concentration values were plotted and R2 was calculated to assess variability. Demographic information, reasoning for the test, specimen type, detection of volatiles and vitreous electrolytes, interval between death and analyses, and cause and manner of death in each case were evaluated.

Results: A total of 54 cases were reported over the study period in the toxicology lab. BHB concentrations ranged from 0.01-5.1 mmol/L (mean 2.40 mmol/L). Preserved iliac, femoral, heart, iliofemoral, subclavian, and antemortem blood samples were analyzed. ME's recorded BHB concentrations on unpreserved blood samples in 7 cases. Concentrations ranged from 0.4-4.8 mmol/L (mean 2.7 mmol/L). Of cases with paired data, 7 of the toxicological concentrations were above ME concentrations and 5 were below with a R2 value of 0.639. Ketoacidosis was listed as a cause of death in 44.44% of the studied cases.

Discussion: Despite a positive correlation, there were notable differences in BHB concentrations between ketone meter readings obtained from unpreserved samples at the morgue and preserved samples at the laboratory. Further research is needed to determine if the differences are due to preservatives or changes in the ketone concentrations over time. This can be better evaluated using a validated GC/MS method, to measure BHB in both preserved and unpreserved blood samples. Once sample suitability is established, if ketone meters are reliable MEs may be able to report BHB levels directly at autopsy.

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[iv] *Mitchell, C. and McCleskey, B., 2010. Utility of a Handheld Blood Ketone Meter as a Postmortem Indicator of Diabetic Ketoacidosis. The American Journal of Forensic Medicine and Pathology [e-journal] 44 (1) pp. 17-20.https://doi.org/10.1097/PAF.0000000000000794.er433gvb.*

Determining Cannabinoid Acetate Analog (Δ^9-THC-O-A, Δ^8-THC-O-A, CBD-di-O-A) Metabolites Using Human Liver Microsomes to Determine the Structure and Relative Appearance of Metabolism

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Introduction: The legality of various cannabinoids and their analogs is complex and varies between federal and state statutes. This is more complex since the Agricultural Improvement Act of 2018 (2018 Farm Bill) federally defined 'hemp'. The cannabinoid acetate analogs: Δ^9-Tetrahydrocannabinol-O-acetate (Δ^9-THC-O-A), Δ^8-Tetrahydrocannabinol-O-acetate (Δ^8-THC-O-A), and cannabidiol di-O-acetate (CBD-di-O-A); are classified as Schedule I controlled substances as they do not occur naturally in cannabis plants and can only be obtained synthetically.

Similar to the synthesis of heroin from morphine, Δ^9-THC-O-A and Δ^8-THC-O-A can be synthesized from Δ^{10}-THC, Δ^9-THC, Δ^8-THC, and CBD by an acetic anhydride reaction. CBD-di-O-A can only be synthesized from CBD. The acetate analogs are formed by the addition of an acetyl group to each hydroxyl group present on the original cannabinoid structures. Although there's little information known about the effects of Δ^8-THC-O-A and CBD-di-O-A, antidotal reports indicate that Δ^9-THC-O-A is called a "spiritual cannabinoid" due to its psychedelic properties producing vivid hallucinations.

These cannabinoid analogs have caused reason for concern due to their increasing prevalence in gummies and vapes. Currently, there is no presented research on how the cannabinoid acetate analogs are metabolized and their detection in biological matrices. Due to the similar synthesis between the acetate analogs and heroin, its suspected to metabolize rapidly in the liver.

Methods: For the metabolism studies, human liver microsomes maintained at body temperature (37°C) were fortified with either a negative control (drug-free), positive control (Δ^9-THC), or a cannabinoid acetate analog (Δ^9-THC-O-A, Δ^8-THC-O-A, or CBD-di-O-A), in triplicate. Analytes were prepared in a solution containing 1 µm of the acetate analog or Δ^9-THC, 5 µL of DMSO, 417.5 µL of deionized water, 50 µL of 1M phosphate buffer pH 7.4, and 15 µL of 5 mM NADPH. Following a 5-minute equilibration of the solutions at 37°C, 12.5 µL of human microsomes (20 mg protein/mL) was added. The metabolic reactions in the positive control and the acetate analogs were terminated with the addition of 200 µL of acetonitrile at 2, 4, 8, 16, and 32 minutes, and the negative control at 2 and 32 minutes. A previously validated high-performance liquid chromatography tandem mass spectrometry method was used to analyze the acetonitrile layer for each acetate analog, its respective plant-based cannabinoid, and its respective hydroxy and carboxy metabolites.

Results: In the Δ^9-THC-O-A solutions, Δ^9-THC-O-A was >25 ng until 16 minutes where this analog was present at <25 ng, Δ^9-THC was >25 ng at all time points except the 16-minute time interval, 11-OH-Δ^9-THC was >25 ng at all time points, and 11-COOH-Δ^9-THC was detectable after 8 minutes at <25 ng. In the Δ^8-THC-O-A solutions, Δ^8-THC-O-A and Δ^8-THC was >25 ng at all time intervals, 11-OH-Δ^8-THC was >25 ng at 4 minutes, and 11-COOH-Δ^8-THC was detectable at 8 minutes at <25 ng. CBD-di-O-A was >25 ng at 2 and 8 minutes and <25 ng at the rest of the time intervals. CBD-di-O-A first metabolized into the newly identified metabolite named CBD-mono-O-A, then to CBD. CBD-mono-O-A was >25 ng until 16 minutes where this metabolite was present at <25 ng. CBD was >25 ng at all time points except the 4-minute time interval. 6-OH-CBD was not detected, 7-OH-CBD was >25 ng after 8 minutes, and 7-COOH-CBD was detectable at 8 minutes at <25 ng.

No cannabinoids were detected at either time point in the negative controls. In the positive controls, Δ^9-THC was >50 ng at all time points except the 4-minute time interval, 11-OH-Δ^9-THC was >50 ng at 2 minutes and increased over time, and 11-COOH-Δ^9-THC was detectable after 8 minutes at <25 ng. There were no signs of degradation in the reagent blanks and no metabolites were detected.

Discussion: Δ^9-THC-O-A, Δ^8-THC-O-A, and CBD-di-O-A all metabolize rapidly but are visually distinct to one another. This indicated that the placement of the double bond on the structural isomers (Δ^9-THC-O-A and Δ^8-THC-O-A) matter as do

the number of acetate groups present on the structure. The acetates metabolites and how rapid they form may be an issue, as it may not be possible to differentiate whether the acetate analog or the plant-based cannabinoid were consumed when testing urine from an individual who reportedly consumed the acetate analog.

2024 Update on Standards Development Activities in Forensic Toxicology

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

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

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

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

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

ASB-published documents that have been added to the OSAC Registry:

- ANSI/ASB 017: *Standard Practices for Measurement Traceability in Forensic Toxicology*;
- ANSI/ASB 036: *Standard Practices for Method Validation in Forensic Toxicology*;
- ANSI/ASB 037: *Guidelines for Opinions and Testimony in Forensic Toxicology;*
- ANSI/ASB 053: *Standard for Report Content in Forensic Toxicology*;
- ANSI/ASB 054: *Standard for Quality Control Programs in Forensic Toxicology Laboratories;*
- ANSI/ASB 098: *Standard for Mass Spectral Data Acceptance in Forensic Toxicology*;
- ANSI/ASB 113: *Standard for Identification Criteria in Forensic Toxicology;*
- ANSI/ASB 119: *Standard for the Analytical Scope and Sensitivity of Forensic Toxicological Testing of Blood in Medicolegal Death Investigations*;
- ANSI/ASB 120: *Standard for the Analytical Scope and Sensitivity of Forensic Toxicological Testing of Blood in Impaired Driving Investigations*;
- ANSI/ASB 121: *Standard for the Analytical Scope and Sensitivity of Forensic Toxicological Testing of Urine in Drug-Facilitated Crime Investigation;*
- ANSI/ASB 152: *Standard for the Minimum Content Requirements of Forensic Toxicology Procedures;*
- ANSI/ASB 153: *Standard Practices for Proficiency Testing for Forensic Toxicology Laboratories;* and

- ANSI/ASB 156: *Guidelines for Specimen Collection and Preservation in Forensic Toxicology*

ASB-published documents that have yet to go through the OSAC Registry approval process:

- ASB 055: *Standard for Breath Alcohol Measuring Instrument Calibration;*

Documents currently in development by the ASB:

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

Documents currently being drafted at the OSAC:

- *Quality Assurance Management Systems in Forensic Toxicology Laboratories*;
- *Standard Method for Blood Ethanol Identifications and Quantitations*;
- *Standard for the Identification and Quantitation of Volatile Chemicals in Biological Fluids;*
- *Best Practice for the Analysis of Carboxyhemoglobin for Forensic Toxicology Laboratories;*
- *Standard Method for Breath Alcohol Subject Testing;* and
- *Human Factors Considerations for Forensic Toxicology Laboratories*

Revisions to existing standards, priorities of new documents or work products, and other highlights.

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

Evaluation of a Case Series Involving N,N-Dimethylpentylone: A Retrospective Study and Trends

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Introduction: The novel psychoactive substances (NPS) market is changing rapidly and continues to pose great challenges for forensic toxicology laboratories, and public health. Since 2018, novel stimulants represent the third largest category among the newly reported NPS in the US. Over the years, stimulants have been reported in death investigations, intoxication events, and emergency room admissions. As soon as the substituted cathinone eutylone was recommended for international monitoring, a shift in the drug market was observed that led to an increase in N,N-Dimethylpentylone (DMP) proliferation. N,N-Dimethylpentylone, also known as dipentylone or beta-keto-dimethylbenzodioxolylpentanamine, is a synthetic cathinone and an isomer of N-ethyl pentylone, a substituted cathinone that was prevalent around 2017-2018. In the US, DMP was first identified in toxicology cases at the end of 2021. Since then, DMP has been the most commonly encountered synthetic stimulant to appear in forensic casework. Providing up-to-date analysis and information regarding the prevalence and use patterns has become crucial to promote increased awareness.

Objectives: The aim of this study was to provide data on the prevalence of DMP in a case series from November 2021 to February 2024 for five Medical Examiner Districts in the state of Florida.

Methods: Postmortem blood specimens were initially screened using gas chromatography-nitrogen phosphorus detection (GC-NPD) and gas chromatography-mass spectrometry (GC-MS) at the Forensic Toxicology Laboratory of the University of Florida, and submitted to CFSRE and/or NMS for confirmation. At the CFSRE, samples were screened using liquid chromatography-quadrupole time-of-flight mass spectrometry (LC-QTOF-MS) and positive samples were reflexed onto a liquid chromatography-tandem quadrupole mass spectrometry (LC-QQQ-MS) confirmatory method. The quantitative method included the novel synthetic cathinones DMP, pentylone, and eutylone, as well as their structural isomers, and the analytical range was 10-1000 ng/mL. DMP was added to the scope of an existing cathinone assay at NMS Labs that already included its metabolite, pentylone. Analysis was achieved using liquid chromatography-tandem mass spectrometry (LC-MS/MS) for detection and quantitation; the reporting range of all analytes was 10-1000 ng/mL.

Results: DMP was detected in 152 postmortem blood samples. The age of the decedents ranged from 19-75 years old (median: 39.0 years; mean: 40.9 years). The majority were male (75.7%), with the race distribution being nearly equal between black and white, 48.7% and 46.7% respectively, with the remaining 4.6% hispanic. The blood concentration range for DMP was 10-3300 ng/mL (median: 130 ng/mL; mean: 266 ± 396 ng/mL). For 7 samples (4.6%), DMP was detected but not quantified as it was below the lower limit of quantitation (LLOQ, 10 ng/mL). The DMP metabolite pentylone was detected in 131 (86.2%) of the 152 cases, with a blood concentration range of 10-710 ng/mL (median: 54.5 ng/mL; mean: 88.3 ± 103 ng/mL). For 27 samples (20.6%), pentylone was below the LLOQ (10 ng/mL). The structurally similar compound eutylone was also detected in 9 cases (5.9%), with a blood concentration range of 10-120 ng/mL (median: 65.0 ng/mL; mean: 65.0 ± 55.0 ng/mL). For 7 samples (77.8%), eutylone was below the LLOQ (10 ng/mL). Polysubstance use with DMP was seen with other synthetic cathinones (6.6%), traditional stimulants (60.5%), fentanyl and its analogs (50.7%), other opioids (30.3%), benzodiazepines (17.8%), cannabinoids (10.5%), and other NPS stimulants (2.0%). In a majority of the cases (65.8%), the case history indicated it was a suspected drug overdose. Moreover, 10 cases (6.6%) involved a motor vehicle crash (MVC), 16 cases (10.5%) involved acts of violence, 5 cases (3.3%) were suicide, and 5 cases (3.3%) involved inmates in prison.

Discussion: Based on the NPS stimulants trends in the illicit drug supply, this study aimed to provide a better understanding of the DMP epidemic in our area of investigation and to promote increased awareness for future strategies of analysis and prevention.

Emergence of *ortho*-Methylfentanyl in Medicolegal Death Investigation Cases from North America

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Background/Introduction: Since the placement of fentanyl analogues in Schedule I of the Controlled Substances Act in 2018 by the Drug Enforcement Administration, novel synthetic opioids structurally dissimilar to fentanyl (e.g., nitazenes) have emerged more commonly in the recreational drug markets with few fentanyl analogues appearing. However, in 2023, *ortho*-methylfentanyl was first detected in drug markets in Canada, specifically in Vancouver and Toronto. *ortho*-Methylfentanyl is a Schedule I drug in Canada as a fentanyl analogue and is Schedule I in the United States. *ortho*-Methylfentanyl is an active mu-opioid receptor agonist that is equipotent to fentanyl. Similar to *para*-fluorofentanyl, *ortho*-methylfentanyl has been identified alone and with fentanyl. It is unknown what caused the addition of *ortho*-methylfentanyl into the opioid supply in Canada and it has not yet been identified in the United States; however, we know the precursor for this drug (*ortho*-methyl 4-anilino-1-boc-piperidine) is available for purchase online. The rapid identification of *ortho*-methylfentanyl linked to overdose and death is of high concern for public health and safety.

Objectives: This study sought to develop an analytical workflow for the identification and quantitation of *ortho*-methylfentanyl in authentic forensic biological specimens. This was achieved by developing and validating a quantitative assay for three methylfentanyl isomers and their metabolites using liquid chromatography tandem quadrupole mass spectrometry (LC-QQQ-MS). The method was applied to blood specimens suspected to contain *ortho*-methylfentanyl following comprehensive drug screening.

Methods: The isomers included in this method were *ortho*-, *meta*-, and *para*-methylfentanyl, and methyl-4-ANPP. Fentanyl-D5 was used as the internal standard. The quantitative range was assessed from 0.1 to 100 ng/mL in blood. 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. Chromatographic separation was achieved on an Agilent InfinityLab Poroshell C-18 120 (1.8 µm, 2.1x150 mm) analytical column using gradient elution. Mobile phase compositions were 5 mM ammonium formate in water (pH 3) and 0.1% formic acid in methanol. The flow rate was 0.4 mL/min, the injection volume was 5 µL, and the column temperature was 60ºC. A fit-for-purpose validation was completed in consultation with ASB Standard 036. Authentic specimens collected for-cause and provided in collaboration with NMS Labs were subjected to the method. Basic demographic and case information were collected, when available.

Results: *ortho*-Methylfentanyl was chromatographically resolved from its *meta*- and *para*- isomers. The method met all validation requirements. The method was applied to 83 cases collected between January 2023 and February 2024 from British Columbia, Canada. Individuals ranged from 19 to 75 years and were primarily male (71%). Brief histories were provided for all cases, 68% describing “sudden death with a history of drug use”. *ortho*-Methylfentanyl was confirmed in all blood specimens with a median concentration of 2.1 ng/mL (mean: 5.8±16 ng/mL, range: 0.1-130 ng/mL). No other methylfentanyl isomers were identified. Methyl-4-ANPP was identified in 81% of cases. *ortho*-Methylfentanyl was most commonly discovered alongside other substances, including fentanyl (88%), *para*-fluorofentanyl (83%), carfentanil (8.4%), methamphetamine (78%), bromazolam (66%), and desalkylgidazepam (11%). The only adulterant identified in this case series was xylazine identified in 7.2% of cases. *ortho*-Methylfentanyl was detected without the presence of other opioids in only 4.8% of cases.

Conclusion/Discussion: The validated assay was successfully implemented for the quantification of *ortho*-methylfentanyl in blood from medicolegal death investigations. o*rtho*-Methylfentanyl is the newest fentanyl analogue to appear in postmortem blood samples with regularity, and its emergence seems similar to that of *para*-fluorofentanyl in the fentanyl supply. Based on experience with prior fentanyl analogues, *ortho*-methylfentanyl could cross over into other drug supplies in North America and beyond. Forensic toxicology laboratories should prepare for the emergence of *ortho*-methylfentanyl and add testing for this drug in forensic casework.

Optimization of generative AI tools for high throughput detection of NPS by retrospective analysis of HRMS data

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Introduction: The illicit drug supply is constantly evolving, causing adverse health outcomes for people who use unregulated drugs, resource strains for forensic laboratories, and challenges for the criminal justice system. While current methods to identify novel psychoactive substances (NPS) through collaboration and information sharing have dramatically improved laboratory testing and surveillance, comprehensive testing for NPS is limited in many jurisdictions and results, when available, may not be timely. Automated processing tools using generative artificial intelligence (GenAI) can be applied to forensic datasets to identify both known and unknown NPS in less time with fewer resources. We previously developed a tool based on GenAI to identify substances from a curated library of 83 NPS using a dataset with more than 12,000 samples as a proof of concept. Further development is in progress to increase the scope of testing by using libraries created through crowdsourcing of known compounds (HighResNPS) and GenAI of theoretical substances (DarkNPS). However, the sensitivity and specificity of this approach must improve before larger libraries can be used.

Objectives: The aim of this study is to evaluate the use of autonomous detection of known and unknown NPS in postmortem toxicology specimens using previously developed tools and identify factors that can improve sensitivity and specificity.

Methods: High resolution mass spectrometry (HRMS) data were acquired using a Thermo Q Exactive HF Orbitrap LC-MS/MS as part of routine postmortem toxicology screening. Data from 1725 samples from suspected drug toxicity deaths spanning 14 months were reanalyzed retrospectively using in-house developed software tools coded in R and Python running in a cluster computing environment. Analysis was performed using a 16,000 CPU core/200 GPU high performance computing platform. Retention times for unknown compounds were predicted using a model based on artificial neural network with the retention times of 300 confirmed substances as training data. Presumptive findings were compared to results obtained from confirmatory testing where available.

Results: In the 1725 samples that were analyzed, 25 distinct drugs were presumptively identified and confirmation tests were available for 9 of these 25 drugs. Sensitivity and specificity values for the most prevalent drugs were: bromazolam (99% and 77%), fluorofentanyl (94% and 80%), and desalkylgidazepam (100% and 92%). Phenethylamines, namely eutylone, N-ethylpentedrone, and α-PHiP were presumptively detected in as many as half of the analyzed samples but were not confirmed. Retention times for the phenethylamines were widely dispersed and were often outside of the theoretical retention time window.

Discussion: An autonomous high throughput tool for retrospectively analyzing HRMS data for NPS was evaluated using postmortem samples. Once optimized, this tool can incorporate larger libraries, including those created with GenAI that may identify drugs that have not previously been characterized. The current screen identified bromazolam, fluorofentanyl, and desalkylgidazepam with high sensitivity but relatively low specificity. The low specificity is likely due to the presence of these analytes in some samples at concentrations below the limit of detection for our confirmatory methods (LOD range: 0.05 – 5 ng/mL). The phenethylamine-containing drugs included in the test library - eutylone, N-ethylpent-

edrone, and α-PHiP - were ubiquitous in preliminary screening with retention times appearing to be randomly distributed. This is not unexpected given that phenethylamine groups are common in endogenous molecules. Future analyses can eliminate many of these false positives by filtering based on theoretical retention times and other criteria. Automated retrospective analysis tools that incorporate GenAI have tremendous potential to mitigate harms associated with NPS distribution and use.

Emergence of Medetomidine as an Opioid Adulterant Encountered with Fentanyl, Xylazine, and Other Substances

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Introduction: The drug landscape in the United States remains complex, multicomponent, and everchanging. While fentanyl is the primary drug linked to opioid-related fatalities, it is rarely seen alone in forensic toxicology cases. Other CNS depressants, such as benzodiazepines and sedatives, are commonly present as adulterants in street fentanyl. Medetomidine, an alpha-2 agonist like xylazine and clonidine, used in both human and veterinary medicine is a synthetically manufactured drug that has recently emerged in drug supplies together with fentanyl and, less commonly, heroin. Medetomidine exists in two enantiomeric forms: dexmedetomidine and levomedetomidine, each with different pharmacokinetic and pharmacodynamic profiles. The effects of medetomidine are noted to include sedation, analgesia, muscle relaxation, anxiolysis, bradycardia, hypotension, hyperglycemia, and hallucinations. The duration of action for medetomidine is reportedly longer relative to xylazine which is concerning from public health and treatment perspectives.

Objectives: The main objectives are: i) to discuss the emergence of medetomidine in North American drug markets, ii) to identified what substances are commonly found along with medetomidine in toxicology specimens, iii) to report on the quantification of medetomidine in blood specimens, and 4) to illustrate which form of medetomidine is present in recreational drug samples.

Methods: Antemortem and postmortem whole blood from individuals suspected of consuming opioid drug products containing medetomidine were collected and analyzed. Blood specimens (0.5 mL) were prepared for analysis using a single-step basic liquid-liquid extraction. Qualitative drug screening was performed by liquid chromatography quadrupole time-of-flight mass spectrometry (LC-QTOF-MS) using a SCIEX X500R. Resulting datafiles were processed against an extensive in-house database of more than 1,190 substances. When available, samples screening positive for medetomidine were forwarded for quantitation of medetomidine, fentanyl, xylazine, and metabolites. Quantitative drug confirmation was performed by liquid chromatography tandem quadrupole mass spectrometry (LC-QQQ-MS) using a Waters TQS. Secondarily, a separate LC-QQQ-MS confirmatory method using an Agilent 6495 was developed to separate dexmedetomidine and levomedetomidine.

Results: Medetomidine first began appearing in samples analyzed by this laboratory in mid-to-late 2023; however, these detections were initially isolated incidents and not tied to greater drug market proliferation. Of note, because the d-medetomidine isomer (dexmedetomidine, Precedex™) is used in human clinical medicine, cases with history of emergency department admissions were reviewed to confirm dexmedetomidine was not administered. Beginning in late April 2024, medetomidine began appearing with regularity in drug materials submitted from Philadelphia and Chicago, and additional toxicology specimens were submitted soon after. By May 2024, medetomidine has been identified in samples collected from at least six states. Thirteen blood specimens linked to an overdose outbreak in Philadelphia were submitted for analysis in May 2024. Specimens originated from individuals hospitalized after suspected exposure to fentanyl adulterated with medetomidine, and symptoms observed included more profound bradycardia than was typical of the fentanyl and xylazine drug supply. Medetomidine was identified in eleven blood specimens, in addition to fentanyl (n=11), xylazine (n=11), cocaine (n=5), methamphetamine (n=3), oxycodone (n=2), PCP (n=1), and bromazolam (n=1). Isomer differentiation indicated that all cases contained both d- and l-medetomidine. The total medetomidine blood concentrations for the initial eleven cases from Philadelphia were mean (±SD) 6.2±5.2 ng/mL, median 4.8 ng/mL, and range 1.2-16 ng/mL.

Discussion: Medetomidine is the latest CNS depressant to appear as an adulterant alongside fentanyl in the recreational drug supply. Medetomidine has been previously reported in Maryland and Toronto; however, recent data show this adulterant is proliferating across the United States and more commonly appearing in forensic toxicology specimens. In cases where medetomidine ingestion is suspected or confirmed, severe adverse effects have been noted, including heightened sedation and profound bradycardia. Forensic toxicology laboratories should begin testing for medetomidine routinely, and forensic toxicologists should be prepared to evaluate and interpret cases involving this new substance.

Prevalence of Psychiatric Medication Use Among Pilots Involved in General Aviation Accidents from FY2019 to FY2023

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Introduction: Recent events have highlighted the underreporting of mental health diagnoses in aviation, sparking discussions on the use of psychiatric medications. Balancing impairment risk versus untreated mental health disorders is a main concern. As of April 2010, airmen with mild to moderate depression treated with one of the following medications: fluoxetine, sertraline, citalopram, or escitalopram are eligible to obtain a special issuance of an airman medical certificate by the Federal Aviation Administration (FAA). As of May 2024, desvenlafaxine, duloxetine, venlafaxine, and bupropion were added to the conditionally acceptable medications list. Use of psychiatric medications outside of these conditionally approved medications is prohibited. Pilots report a fear of being restricted from flying as a reason for failing to disclose mental health diagnosis and medications; thus, leading to an unknown number of pilots flying while taking disallowed medications.

Objective: To evaluate the incidence of psychiatric medication(s) use in general aviation accidents from FY2019 to FY2023.

Method: General aviation accident cases submitted to the laboratory from October 2018 to September 2023 (FY2019 – FY2023) were data mined for the presence of psychiatric medications, including antidepressants, antipsychotics, anxiolytics, and attention-deficit/ hyperactivity disorder (ADHD) medications. Biological samples from aviation accidents underwent full toxicological testing and only psychiatric medications confirmed by gas chromatography mass spectrometry or liquid chromatography tandem mass spectrometry were included. Midazolam and lorazepam were not included due to potential administration during hospital treatment. Ketamine was not included when present with fentanyl, midazolam, and/or lorazepam.

Results: From FY2019 to FY2023, 1,262 pilots were involved in an aviation accident with subsequent toxicology testing, of which, 127 tested positive for at least one psychiatric medication. Pilots that tested positive for psychiatric medication(s) exhibited similar demographics to that of the pilot population that were negative for psychiatric medications. The average age of pilots positive for a psychiatric drug was 57 years old with a range from 18-84 years old. Approximately 91% of the pilots positive for a psychiatric drug were male.

Out of the 127 pilots positive for at least one psychiatric medication, 76 pilots held a current Airman's Medical Certificate. Of which, 82% were positive for an antidepressant medication. One pilot reported the use of their psychiatric medication to the Airman's Medical Examiner (AME). Twenty-three other pilots with medical certificates were positive for an antidepressant on the FAA's conditionally approved medications list but failed to report the medication to the AME. Medications taken by pilots with an Airman's Medical Certificate extended beyond the antidepressants approved during the examined years, and included trazodone, tramadol, venlafaxine, amitriptyline, duloxetine, bupropion, doxepin, vilazodone.

Within the population of pilots with current Airman's Medical Certificates, poly-psychiatric medication use was observed in 20 pilots. Of the cases of poly-psychiatric medication use, a combination of antidepressants and anxiolytics was the most prevalent combination over the 5-year period with 7 cases out of 20. Second most prevalent poly-psychiatric occurrence was a combination of multiple antidepressant medications (4 cases).

Discussion: From FY2019 to FY2023, 10% of pilots involved in general aviation accidents with subsequent toxicology analysis tested positive for at least one psychiatric medication. Within this pilot population, the use of psychiatric medications is underreported and extends beyond medications on the FAA's conditionally approved list.

Update on Acute and Chronic Oral Dosing of Cannabidiol (CBD) With and Without Low Doses of Delta-9-Tetrahydrocannabinol (Δ9-THC)

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Introduction: Hemp products that predominantly contain cannabidiol (CBD) and low concentrations of delta-9-tetrahydrocannabinol (Δ9-THC) are legal in many jurisdictions and are widely used for health or wellness purposes. Insufficient research has been conducted to determine the threshold of Δ9-THC dose in these products that can impact safety or that result in positive drug testing outcomes.

Objectives: Determine the behavioral pharmacology and toxicology of acute and repeated oral cannabidiol (CBD) administration with and without low doses of Δ9-THC that approximate amounts likely to be found in "full-spectrum" retail hemp products based on product testing research, the 0.3% THC threshold for hemp, and the 100 mg/mL concentration of CBD selected for this study (equivalent to Epidiolex).

Methods: Healthy adults with no self-reported past month cannabis use and negative for THC metabolites in urine at baseline were randomized to self-administer 1 mL medium-chain triglyceride (MCT) oil that contained 100 mg CBD and either 0, 0.5, 1, 2, 2.8 or 3.7 mg Δ9-THC twice daily for 14 days. The drug products were compounded in MCT oil by the Johns Hopkins Investigational Drug Service (IDS) research pharmacy from purified, hemp derived, CBD and pure Δ9-THC. Initial dose exposure occurred during an 8-hr laboratory session during which subjective drug effects, vitals and cognitive performance, including Digit Symbol Substitution Task, Paced Serial Addition Task, Divided Attention Task and the DRiving Under the Influence of Drugs (DRUID) App, were assessed and blood, urine, and oral fluid were collected for analysis. Outpatient dosing (14 days) was monitored via video surveillance to ensure compliance with the instructed dosing regimen (additional Δ9-THC exposure cannot be ruled out, but is unlikely based on an absence of cannabinoids after a 1- week washout) and additional assessments collected during brief visits on Days 2, 7, 14, and 21 (1-week washout).

Results: There was no difference between Δ9-THC conditions (0 to 3.7mg per dose) on subjective drug effects or cognitive performance, indicative of no or very low levels of interoceptive drug effects.

At least one positive urine test (screening via immunoassay ≥50ng/mL; confirmation via LC-MS/MS ≥15 ng/mL THC-COOH) was observed for 29 of 36 participants assigned to ≥1 mg Δ9-THC dose conditions; 4 of 10 in the 0.5 mg Δ9-THC dose condition had a positive urine test. Of 28 participants receiving ≥2 mg Δ9-THC, 15 had at least one positive urine test after a single dose.

All participants receiving ≥2 mg Δ9-THC had at least one positive oral fluid test (LC-MS/MS ≥2 ng/mL Δ9-THC); 8 of 9 participants in the 1 mg Δ9-THC dose condition and 5 of 10 in the 0.5 mg Δ9-THC dose condition also had at least one positive oral fluid test.

Blood Δ9-THC concentrations were low across doses; only 4 samples exceeded 2 ng/mL Δ9-THC. All samples, except for one urine, were negative after the 1-week washout.

Discussion: Acute and chronic administration of 100 mg CBD with 0.5-3.7 mg Δ9-THC produced zero to mild subjective drug effects and did not impact cardiovascular or cognitive endpoints. Positive urine and oral fluid drug test results are likely after acute Δ9-THC doses of 2 mg or higher and chronic use of 1 mg Δ9-THC or higher. At the lowest dose of Δ9-THC (0.5 mg), occasional positive urine tests were encountered.

Hemp-derived or semisynthetic cannabinoids: pharmacology of isomers and how it matters for the harm potential of seized drugs

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Introduction: The introduction of the 'Farm Bill' (2018) in the US, differentiating *hemp* (less than/equal to 0.3% D^9-tetrahydrocannabinol (THC), dry weight) from *marijuana*, created what is called 'a legal loophole' in the cannabis industry. Cultivation of industrial low-THC hemp created an oversupply of cannabidiol (CBD)-rich products, which was then used as starting material to diversify the markets by producing 'hemp-compliant' THC-analogs. These analogs include D^8-THC, THC-homologs, hexahydrocannabinol (HHC) and other semisynthetic cannabinoids that can be derived by chemical conversion of hemp-products.

Objectives: This study aimed to determine the potential for abuse or harm (as assessed by activation of the CB_1 receptor) of purified forms of phytocannabinoids and semisynthetic cannabinoids, to allow prioritization of legislative efforts and research endeavors for the monitoring of these compounds in control programs.

Methods: The potential for abuse was evaluated for a large panel (n=30) of commercially available THC-homologs (D^8-THC- and D^9-THC-homologs with a 1- to 8-carbon alkyl chain), -isomers (e.g. *exo*-THC) and -analogs ((9)S- and (9)R-HHC and -HHCP), that might possibly be derived with semisynthetic procedures. This study focused on the compounds' potential to activate the CB_1 receptor, relative to D^9-THC, the primary psychoactive compound in *cannabis*. A cell-based NanoBiT® (Promega) b-arrestin2 recruitment assay was used to assess the intrinsic receptor activation potential of the CB_1 receptor, which is the prime receptor responsible for the psychoactive effects of *cannabis*. In addition, seized drug samples of HHC material (Belgium) were investigated with liquid chromatography coupled to high resolution mass spectrometry (LC-HRMS) to assess the relative composition of different diastereomers, followed by an assessment of how differences in the relative diastereomer composition impacted biological activity.

Results: THC-homologs with longer alkyl chain lengths (THCH, hexyl-tail; THCP, heptyl-tail; THC-C8, octyl-tail), together with THC-analogs HHC and hexahydrocannabiphorol (HHCP) and the THC-isomer *exo*-THC, were identified as compounds with higher CB_1 receptor activity than D^9-THC, based on either potency (EC_{50}) or efficacy (E_{max}). Other THC-isomers showed lesser potential for CB_1 activation, making them less prone to widely emerge as semisynthetic cannabinoids on the recreational drug market. In general, the pharmacology of stereoisomers (S *versus* R) was pronouncedly different, with one isomer showing higher CB_1 activity than the other, as exemplified for HHC and HHCP stereoisomers. The relevance of this was demonstrated in seized HHC drug products that showed various relative compositions of 9(S)-HHC (21-47%) and always contained more 9(R)-HHC, which was characterized as the epimer with the highest CB_1 activity. In literature it has been reported that different epimeric compositions of HHC can be obtained through varying semisynthetic production routes/conditions. Different batches of HHC material could be distinguished based on the epimeric composition, which was consistent with the labeling of the different HHC-containing recipients. Bioactivity-wise, based on the evaluation of different epimeric mixtures of reference standards of 9(R)- and 9(S)-HHC, a decreased relative abundance of the 9(S)-epimer was empirically shown to lead to an increased potency of the epimeric mixture. Hence, HHC material with increased 9(R)-HHC abundance can anticipated to be more psychoactive.

Discussion: Multiple derivatives of THC show higher intrinsic CB_1 activation potential than D^9-THC. Many of these have already emerged on recreational drug markets (e.g. HHC, HHCP, THCP), others might be anticipated to share the same fate and may be prioritized for (legal) follow-up. Monitoring these semisynthetic cannabinoids is encouraged as the dosing (related to the potency studied here) and the relative composition of stereoisomers is hypothesized to greatly impact the psychoactivity and harm potential of these drugs, relative to D^9-THC products. Additionally, determination of the epimeric composition of drug products could be useful for batch identification.

Investigation into CBD user urine testing positive for THC in a drug screening kit

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Introduction: Cannabidiol (CBD), one of the active phytocannabinoid components in cannabis, is unregulated in most countries including Japan because it does not have the same psychoactive effects or exhibit dependence effects that could lead to abuse like Δ9-tetrahydrocanabinol (THC). In recent years CBD has become a popular ingredient in many health foods and cosmetics, and has also been approved as a prescription drug in many countries. The CBD market has thus rapidly expanded as a familiar and easily accessible substance.

We report a case in which a CBD user urine tested positive for THC in a drug screening kit. A 20s male was hit by a car and was transported to the emergency department (ED), was then intubated and admitted for treatment and observation. Upon initial clinical assesment at the ED the patient's blood alcohol level was 354 mg/dL and he tested positive for THC using drug screening kit IVeX-screen®M-1 (BioDesign, Inc., Tokyo, Japan), hence marijuana use was suspected. After regaining consciousness, the patient was extubated and interviewed where he denied drug use but commented on his habitual use of commercial CBD oil.

Objectives: To perform an investigative follow-up instrumental analysis of a habitual CBD user's urine that resulted in a THC false-positive in an immunoassay drug screening kit.

Methods: Urine samples collected at the ED (0, 9, 17, 23, and 47h from ED admission) were prepared by acetonitrile protein precipitation prior to instrumental analysis. Screening for CBD and its metabolites, as well as THC and its metabolites were performed on a X500R QTOF (AB Sciex, Framingham, MA, USA) mass spectrometer coupled to ExionLC™ using a CORTECS T3 Column (120Å, 2.7 µm, 100 x 2.1 mm; Waters, Milford, MA, USA) under gradient elution using (A) water, 0.1% formic acid and (B) acetonitrile mobile phases and positive ESI detection in the Scan+ IDA mode. For quantitative analysis, a QTRAP4500 mass spectrometer coupled an ekspert™ ultraLC 100-XL system (AB Sciex, Framingham, MA, USA) was used in the multiple reaction monitoring (MRM) mode, using the same column and LC parameters used for screening.

Results: THC and THC metabolites were undetected upon urinary screening by LC/Q-TOFMS. Unchanged CBD was also undetected but the oxidative and glucuronide (presumptive) metabolites were detected. Preliminary quantitative analysis results were 5-78 ng/mL for 7-COOH-CBD, and below the limit of quantitation for 7-OH-CBD.

Discussion: Structural estimation through accurate mass analysis by Q-TOFMS showed that the metabolites detected in the patient's urine were the oxidative metabolites (7-OH-CBD and 7-COOH-CBD) as well as the glucuronide conjugate metabolites (CBD-glucuronide and 7-COOH-CBD-glucuronide). Since standard reference compounds for the glucuronide conjugates were not available, data were compared with previously obtained animal experiment data. Quantitated 7-OH-CBD and 7-COOH-CBD concentrations were far below the cutoff concentrations to produce false-positives in IVeX-screen's cross-reactivity datasheet (100,000 ng/mL for both compounds). It is therefore likely that the false-positive is due to CBD metabolites that are unlisted in the cross-reactivity data, which we presume to be the glucuronide conjugates. Further investigation is currently underway to evaluate the cross-reactivity of glucuronide metabolites of CBD on commercial drug screening kits available in Japan.

Marijuana use was suspected from the drug screening kit testing positive for THC, but later patient interview and toxicological analysis results revealed that CBD metabolites likely caused the false positive THC result. With the recent expansion of CBD distribution, cases like these are likely to occur more often in the future, and caution is needed when interpreting on-site drug screening kit results.

In Vitro Formation of 6-Monoacetylmorphine (6MAM) in Oral Fluid Post Collection in the Presence of Morphine and Aspirin

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Introduction: Analysis of oral fluid for 6-monoacetylmorphine (6MAM) detection offers valuable insights into heroin and morphine use. Presence of both 6MAM and morphine indicates heroin use. Presence of morphine alone suggests pharmaceutical use or poppy seed ingestion. Detection of morphine alone may also indicate heroin use due to morphine's longer detection window compared to 6AM; however, a morphine-only result is frequently observed among pain management patients who use morphine or codeine for treatment. In addition to prescribed medications, daily ingestion of acetylsalicylic acid (aspirin) is also common among pain patients and elderly individuals. Naso-Kasper et al (2013) documented in vitro formation of 6MAM and 3MAM in gastric fluids fortified with morphine and aspirin and subsequently identified 3 in >10,000 urine specimens where this conversion may have happened. It is unclear if aspirin intake may play a role in unusual 6MAM oral fluid results.

Objectives: The primary objective of this study was to quantitatively evaluate the possibility and extent of morphine conversion to 6MAM in Quantisal™ oral fluid samples with aspirin under extreme temperature conditions.

Methods: Oral fluid specimens from a healthy volunteer donor were subjected to a standardized incubation protocol to simulate extreme storage conditions (4 days storage at 37°C) that may arise from delayed receipt at the testing laboratory. To investigate the influence of aspirin on the conversion of morphine to 6MAM, a single pulverized aspirin tablet (Bayer®, 325mg) was added to 1mL of oral fluid, which was fortified with 250 ng/mL morphine, and then diluted with 3mL Quantisal™ device buffer to account for the 4-fold dilution factor from standard collections. All calibrators and quality control (QC) samples were also diluted 4-fold. To assess baseline conditions, negative controls containing water and Quantisal™ buffer devoid of oral fluid were also included.

All experimental and quality control samples were replicated in triplicate to ensure robustness and reproducibility of the findings. A multi-analyte panel validated according to CAP/CLIA guidelines and utilizing solid-phase extraction and liquid chromatography-tandem mass spectrometry was used to quantify morphine, 6MAM, and other relevant opioids in this study. For morphine, the limit of quantification (LOQ) was 1.0 ng/mL and cutoff for positivity was 2.5 ng/mL. For 6MAM, LOQ was 0.5 ng/mL and cutoff was 1.0 ng/mL. Verification of analytical separation between 6MAM and 3MAM was achieved using a 3MAM-only QC at 1.25 ng/mL.

Results: Analysis of negative controls and fortified morphine samples without aspirin yielded no detectable levels of 6MAM. Fortified specimens containing morphine and aspirin stored for 4 days at 37°C exhibited measurable concentrations of 6MAM. Specifically, the average (± standard deviation, range n=3) observed 6MAM concentrations were 0.47 ng/mL (± 0.17, 0.45-0.49 ng/mL) in water, 1.34 ng/mL (± 0.14, 1.22-1.48 ng/mL) in negative buffer, and 1.33 ng/mL (± 0.18, 1.14-1.46 ng/mL) in oral fluid donor samples. The 6MAM concentrations observed in water were all just below the 0.5 ng/mL LOQ but within the validated accuracy and precision of this value; additionally, all water concentrations were well below the 1 ng/mL cutoff. These findings indicate an approximate 0.5% conversion rate of morphine to 6MAM in oral fluid samples supplemented with extremely high aspirin concentrations (having an entire aspirin pill present in 1mL of expectorated oral fluid would be unusual). A 3MAM chromatographic peak was observed in all specimens in which 6MAM was observed.

Discussion: We confirmed the potential for morphine to 6MAM conversion in oral specimens under extremely high aspirin concentrations and temperature (37°C) storage conditions. We also confirmed that the presence of a 3MAM peak can be used to distinguish this conversion from heroin use. More studies are required to assess the relevance, if any, of this conversion in everyday testing. The oral fluid aspirin concentrations achieved in this study are unknown. This study represents an extreme dosing scenario, and not a steady state achieved from typical daily high-dose aspirin.

Synthetic Stimulant of the Southeast – Evaluating Continued Prevalence of N,N-Dimethylpentylone

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Introduction: N,N-Dimethylpentylone is a synthetic cathinone of the beta-keto-methylenedioxyamphetamine subclass encountered as a novel psychoactive substance. Like other entactogens, it produces mixed stimulatory and hallucinogenic effects. A wide range of adverse effects have been described following synthetic stimulant use, including agitation, elevated pulse rate, confusion, seizure, and psychosis. In 2023, N,N-Dimethylpentylone was the most commonly detected novel synthetic stimulant in toxicological casework submitted to NMS Labs. Positivity may be correlated with geographic location, with more than half of the positive casework originating from the state of Florida, based on NMS Labs data. Despite its recent prevalence, toxicological interpretation remains challenging due to limited knowledge its effects.

Objectives: The purpose of this study is to summarize N,N-Dimethylpentylone concentrations from a series of cases submitted to a reference laboratory for toxicological analysis in medical examiner death investigation casework. Additionally, this study aims to provide further information on concentration ranges with context specific information to provide the toxicological community with more data on this emerging substance.

Methods: Data were extracted from NMS Labs' (Horsham, PA) Laboratory Information Management System for all cases received from January 1, 2023 to April 30, 2024 from the Florida 4th District Medical Examiner's Office (Jacksonville, FL). Cases that were positive during confirmatory testing for N,N-Dimethylpentylone were classified as postmortem (PM) or antemortem (AM) based on sample type tested (hospital specimen vs autopsy). The mean, median and concentration ranges for each group were determined.

Results: 57 cases were confirmed as positive in this study for N,N-Dimethylpentylone during this timeframe. The average patient age involved in this study was 37 years old and the sex was predominantly male (45/57 cases). Confirmation testing was performed by LC-MS/MS with a lower limit of quantitation of 10 ng/mL. For hospital specimens classified as AM (n=10) the concentrations ranged from 50 to 550 ng/mL (mean: 200 ng/mL, median: 120 ng/mL). For other specimens where PM blood was tested (n=57) the concentrations ranged from 10 to 100000 ng/mL (mean: 2700 ng/mL, median: 440 ng/mL). Pentylone, a reported metabolite, was positive in all but seven cases at corresponding concentrations lower than N,N-Dimethylpentylone, which is consistent with other published works. Pentylone was present below the lower limit of quantitation in those seven cases. The most commonly detected co-findings were delta-9-THC (n=16), ethanol (n=11), fentanyl (n=6), methamphetamine (n=5) and cocaine (n=5).

Discussion: 20 cases were identified in which N,N-Dimethylpentylone was the only confirmed drug reported as positive with comprehensive toxicology testing; the majority involving homicides or suicides. A concentration of 1200 ng/mL in iliac PM blood was reported in a case involving homicide by gunshot wound to the head. The decedent in this case was reported to have used an unknown drug then was observed to be paranoid and aggressive shortly afterward.

In one case of a suspected overdose, a 28-year-old female, presented to the emergency department in the afternoon with complaints of a headache and visual disturbances. She admitted to using "Molly" the night previously. Blood was taken in the hospital shortly after admission. Cerebellar hemorrhage was seen on imaging. The only significant findings in the case were N,N-Dimethylpentylone and pentylone in antemortem blood reported at 150 and 52 ng/mL, respectively.

N,N-Dimethylpentylone detection continues in death investigation casework, however its role in death or cannot be determined solely from the concentration in the case, and interpretation remains challenging. Further investigation in postmortem casework and studies involving other populations, such as driving under the influence (DUID), is needed to provide further context and insight on this emerging stimulant.

Development of a Semi-Quantitative Liquid Chromatography Tandem Mass Spectrometry Method for 62 Drugs in Umbilical Cord Tissue

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Introduction: In utero drug exposure is a significant public health issue and is a threat to the well-being and normal development of the neonate. Identifying neonates exposed to drugs provides opportunities for management of drug intoxication, dependence, withdrawal, and long-term needs into childhood. The most common specimen types for newborn drug testing are meconium and urine. However, umbilical cord tissue (UC) has advantages that make it an ideal matrix for assessing fetal drug exposure, such as ease of collection, large specimen volume, even drug distribution throughout cord, and a long drug detection window. Developing a large, comprehensive pain management drug panel in UC with the inclusion of a prevalent sedative, xylazine, would provide clinical utility for physicians assessing fetal drug exposure.

Objectives: Develop a comprehensive liquid chromatography tandem mass spectrometry (LC-MS/MS) method for semi-quantitative UC analysis.

Methods: Extraction optimization was conducted using randomly selected screened-negative remnant UC specimens fortified with 62 drugs including anticonvulsants (2), antidepressants (2), barbiturates (2), benzodiazepines (17), muscle relaxers (2), opioids (22), over-the-counters (1), sedatives/dissociative anesthetics (4), stimulants (8), cotinine, and xylazine. A linear 4-point calibration model was utilized to establish a narrow quantitative linear range focused around a cutoff. All drugs were then fortified in UC at the cutoff.

Weighed UC specimens (0.5-0.6 g) were homogenized in extraction buffer (50% methanol in water) fortified with internal standards (ISTD). Tissue mixtures were centrifuged at 3,200 relative centrifugal force for 10 minutes. Supernatants were diluted and hydrolyzed using beta-glucuronidase, followed by automated solid phase extraction (SPE). Analytes were chromatographically resolved using a biphenyl column with a 11.4-minute binary gradient composed of 10 mM ammonium formate in water and 0.1% formic acid in 75:25 methanol: acetonitrile (v: v). Data were collected with a Shimadzu LC20ADXR multiplexing system coupled to SCIEX 6500+ MS. All analytes were monitored using 2 transitions and 1 ion ratio. One transition was used for each ISTD. Preliminary validation experiments were determined from CLSI, ASB, and internal requirements.

Results: For all analytes, limits of quantitation (LOQs) ranged from 0.05 ng/mL to 6.250 ng/mL and upper limits of linearity (ULOLs) were 12-fold higher than LOQs. Cutoff concentrations were administratively set at 4-fold LOQ. Compared to neat samples, area recoveries ranged from 18.3% to 81.9% (average 59.1%), indicating matrix effects. However, the internal standards were able to correct for all recovery variation, with all calculated concentrations in UC within 25% of neat concentrations. Fortified samples demonstrated acceptable accuracy with bias from -16% to 8.6% (average 3.8%). Imprecision (% CV) ranged from 0.9% to 13.3% (average 4.9%). No carryover was observed with drug levels up to ULOL levels. No clinically significant interferences were observed with 123 common prescription and nonprescription drugs (e.g. amphetamines, opioids, benzodiazepines, etc.) spiked in negative specimens. To verify the method, 45 remnant UC specimens were tested, and the results were consistent with the original results (40 positives for ≥1 drug and 5 negatives). The positivity rates in descending order were 60.0% for cotinine, 51.1% for opioids, 26.7% for stimulants, 8.9% for sedatives/dissociative anesthetics, 6.7% for xylazine, 2.2% each for anticonvulsants, barbiturates, and benzodiazepines, and 0% for the remaining drugs. For xylazine, concentrations ranged from 1.48 ng/mL to 4.39 ng/mL; fentanyl and norfentanyl were observed in all xylazine-positive specimens.

Discussion: A high-throughput LC-MS/MS method was developed to detect 62 analytes, including prevalent drugs, in UC utilizing automated SPE extraction. Drug prevalence was studied in a small-scale remnant specimen population, and the results were verified with original data. This method provides extensive information on fetal drug exposure to aid physicians and clinicians in addressing associated health risks and legal concerns.

Rapid Quantitative Screening of 16 Synthetic Cannabinoids in Urine Using DART-MS Analysis

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Introduction: Immunoassay-based (IA) detection for drugs of abuse is commonly used as an initial screening step in urine-based drugs testing due to rapid generation of results and ease of automation. However, IAs suffer from issues with cross-reactivity leading to false positives, requiring costly and time-consuming chromatography-based confirmatory testing. As a more selective alternative, Direct Analysis in Real Time-Mass Spectrometry (DART-MS) provides quantitative screening results reducing or eliminating false positives compared to IA-based drug screening. In this work, we report the development of a rapid, screening approach for 16 synthetic cannabinoids in urine: (4-cyano-CUMYL-BUTINACA (1), 4-fluoro ABUTINACA N-(4-hydroxybutyl) metabolite **(2)**, 4-fluoro MDMB-BUTICA **(3)**, 4-fluoro BUTICA butanoic acid metabolite **(4)**, 4-fluoro MDMB-BUTINACA N-butanoic acid metabolite (5), 5-fluro ADB metabolite **(6)**, 5-fluoro MDMB-PICA **(7)**, 5-fluoro MDMB-PICA metabolite **(8)**, ADB-4en-PINACA (9), MDMB-4en-pinaca butanoic acid metabolite **(10)**, ADB-BINACA **(11)**, ADB-BUTINACA **(12)**, ADB-HEXINACA (13), AMP-4en-PINACA **(14)**, JWH 018 N-pentanoic acid metabolite **(15)**, MDMB-CHMICA metabolite **(16)**). This DART-MS screening method successfully measures the targeted synthetic cannabinoids in 96 samples at a rapid throughput of 23 seconds per sample.

Objectives:

- This work describes the development of a novel analytical method aimed at rapidly screening for synthetic cannabinoids in urine
- Rapid quantitative screening for 16 synthetic cannabinoids using DART-MS provides a more sensitive and selective alternative when compared to immunoassay-based screening.
- This work compares quantitative data between LC-MS and DART-MS using samples confirmed positive for one or more of the listed target analytes.

Methods: For method development, triplicate calibration series were prepared by spiking certified drug-free urine with standards **1-16** (0.1-2500 ng/mL) using deuterated AB-PINACA as an out-of-specification standard. Hydrolysis was performed by adding 50 µL Kura enzyme to 500 µL pre-spiked certified aliquots of drug-free urine followed by a 20 minute incubation at room temperature. After hydrolysis, 500 µL 0.1 M Borax buffer (pH=10.4) and 2.5 mL 30:70 (ethyl acetate:n-chlorobutane) were added to each sample followed by a 30 second agitation. Samples were centrifuged at 4000 RPM for 10 minutes and the organic layer was transferred to glass vials and evaporated to dryness under N_2 at 40°C followed by reconstitution in 100 µL MeOH. Reconstituted samples were vortexed and 2 µL aliquots were transferred onto a Bruker DART QuickStrip HTS-96 screen and dried under N2 gas at 40°C for 15 minutes. For analysis, the prepared QuickStrip-HTS 96 screen was loaded onto the DART-TQ-Plus (Bruker Daltonics) triple quadrupole mass spectrometer foranalysis. Recovery was determined in triplicate using certified drug-free urine at 2 levels for each analyte within the linear range of each calibration series. Results were validated against LC-MS using 20 urine samples confirmed as positive for one or more analytes sourced from DrugScan, Inc.

Results: DART and MS parameters were optimized for sensitivity, precision, reduced helium consumption, and fast analysis time. With DART gas temperature and grid voltage optimized at 300°C and 50 V, respectively, unique MS/MS transitions, collision energies, and MS scan times were successfully identified for **1-16**. DART-MS analysis of the synthetic cannabinoid panel resulted in good linear correlation of $R^2 > 0.99$ for all measured analytes and a recovery between 89 and 110% for all 16 analytes across defined calibration ranges. The reported lower level of quantitation (LLOQ) for all analytes is at or below common IA screening cutoff values of between 0.1 to 5 ng/mL for the synthetic cannabinoid panel. In a representative cross-validation plot of urine samples (n=20) the butanoic acid metabolite of the butanoic acid metabolite of MDMB 4-en PINACA concentrations ranged from undetectable to 362 ng/mL with a mean value of 170.5 ng/mL ± 15.2. DART-MS measurements were well correlated with LC-MS measurements (R^2=0.995, $p<0.05$) with a slope of near unity at 0.95. These results indicate that this rapid chromatography-free workflow using DART-MS for quantitative screening is sufficient to detect all 16 analytes at or below the common cutoff values without the high rate of false

positives associated with IA based screening approaches.

Discussion: The results presented demonstrate the suitability of the DART-MS workflow as a rapid, quantitative, and selective alternative to conventional IA-based urine screening by offering a quantitative method with the benefits of minimizing false positives typically associated with IA based screening, avoiding costly and unnecessary chromatography-based confirmatory testing.

Determination of Drugs and Metabolites in Oral Fluid: Comparison of Different Extraction Procedures

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Introduction: Oral fluid has been gaining importance in forensic and clinical toxicology for roadside, workplace and/or drug abstinence monitoring programs, among others. Oral fluid has many advantages over other matrices (blood, urine), like non-invasive collection, reduced risk of tampering, and short detection window reflecting recent drug use. Its main disadvantage is the limited sample volume (<1 mL) and, therefore, sensitive methodologies with a wide panel of drugs are required. However, comprehensive methods that include drugs with different chemical properties, such basic substances (opioids, cocaine, amphetamines) and neutral/acidic compounds (delta-9-tetrahydrocannabinol, THC) are scarce.

Objectives: To develop an analytical method for the determination of 19 drugs and metabolites, including basic drugs (opioids, stimulants, MDMA, PCP, benzodiazepine), and THC, in oral fluid in NeoSal® buffer by liquid chromatography tandem mass spectrometry (LC-MS/MS), and to compare the most common extraction methods in toxicological laboratories, liquid-liquid extraction (LLE), solid phase extraction (SPE), and an alternative technique, supported liquid extraction (SLE). The best procedure was the one that yielded best sensitivity, specificity, robustness and time and cost efficiency.

Methods: All the methods used 1 mL of oral fluid-NeoSal buffer (0.25 mL oral fluid and 0.75 mL buffer). In the optimized LLE, 1 mL of isopropanol was added to the sample, and after vortexing, samples were extracted twice with hexane:ethyl acetate (30:70). The best SPE procedure employed reversed phase cartridges. Samples were loaded at pH 10, and after washing with water:methanol (95:5) and water:methanol:ammonium hydroxide (70:29.5:0.5), the final elution was performed with dichloromethane:isopropanol (75:25). The SLE was investigated with different cartridges, loading volumes, and elution solvents. The best results were obtained loading 1 mL of sample and using 2 x 900 µL of hexane: ethyl acetate (30:70) for elution. The extracts from all extraction procedures were fortified with HCl:MeOH (1:99) before evaporation under nitrogen to avoid amphetamines loss. After reconstitution in mobile phase, the analysis was performed by LC-MSMS, in gradient mode using a C18 column (1.7 µm, 2.1x100 mm), 0.1% formic acid in water (A) and in acetonitrile (B) as mobile phase, and 0.3 mL/min flow rate. Data was acquired in MRM mode in ESI +, monitoring 2 transitions per compound. Deuterated analogs were used as internal standards. The methods were validated following the ANSI/ASB Standard 036 guidelines.

Results: For all procedures, linearity ranged from the limit of quantification (LOQ) of 1 to 200 ng/mL, except for methadone (0.5-20 ng/mL) and PCP (0.5-100 ng/mL). LLE and SPE LOQ (n=9) showed acceptable bias (within ±20%) and imprecision (<20%), but SLE LOQ imprecision failed for 14 compounds. Acceptable bias and imprecision (n=15) were observed for low (3 ng/mL), medium (15 ng/mL) and high (150 ng/mL) QC samples for all procedures. All compounds had extraction efficiencies above 50% for SPE, 16 for LLE and 6 for SLE. Regarding matrix effects, most of compounds showed ion suppression in SPE (11), LLE (13), and SLE (19). In all procedures, THC showed ion enhancement above 100%. No carryover neither interferences were observed with any procedure. As additional observations, SLE extracts took longer (2x) to evaporate, and the SLE extracts contaminated the ion source, producing desolvation line clogging.

Discussion/Conclusion: We performed three method validations and provided three alternatives to test oral fluid for drugs and metabolites, including THC in the drug panel. These methods used 1 mL of oral fluid-buffer sample, which contained only 0.25 mL of neat oral fluid, achieving a 1 ng/mL LOQ. LLE was determined to be the best method due to the sensitivity, robustness, and costs compared to SPE and SLE.

S60

Prevalence and Analysis of Xylazine in Postmortem Casework in Jefferson County, Alabama

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Introduction: Xylazine is a potent sedative spreading within the illicit drug supply in the U.S. Xylazine is almost always seen in combination with other illicit drugs, primarily fentanyl, which increases the chance of overdose. As a secondary amine, xylazine is prone to poor peak shape by gas chromatography (GC) and is a therefore a potential candidate for peak improvement by derivatization.

Objectives: The first objective of this research was to determine the prevalence of xylazine in Jefferson County Coroner/Medical Examiner Office (JCCMEO) cases from January 2019 to January 2024 by evaluating existing toxicology and pathology data. The second objective was to improve xylazine's chromatography by developing and validating a method for detecting and quantitating xylazine at low concentrations by SPE, derivatization and GC with mass spectrometry (MS).

Methods: The JCCMEO database was interrogated for xylazine positive cases over the 5 year period from January 2019 to January 2024 and downloaded to excel spreadsheets which were deidentified. Data included toxicology results, demographic information, and cause and manner of death for each case. The data was analyzed to evaluate xylazine concentrations, demographic trends, post-mortem information, and other drugs co-detected with xylazine.

To successfully quantitate xylazine, derivatization with BSTFA+10 % TMCS was included in the method development and validation. Validation was carried out according to ANSI/ASB Standard 036 including establishing a linear range, bias and imprecision tests, interference testing, dilution integrity, carryover, stability, recovery, limit of detection (LOD), limit of quantification (LOQ), and matrix matching. Instrumental analysis was conducted on an Agilent 8860 Gas Chromatograph (GC) System with 5977C MSD. Seventeen xylazine positive cases saved by the UAB Forensic Toxicology laboratory for this research were tested using this method.

Results: There were 198 xylazine positive cases during the study timeframe. Xylazine cases in Jefferson County peaked in 2022 and have since been declining. The most frequently seen drugs with xylazine included (1) fentanyl, 98% (2) cocaine, 39%, and (3) methamphetamine, 37%. The most frequently seen combinations of these drugs from January 2019-January 2024 included (1) xylazine, fentanyl, and cocaine; (2) xylazine, fentanyl, and methamphetamine; (3) xylazine and fentanyl; and (4) xylazine, fentanyl, cocaine, and methamphetamine. A method for analyzing xylazine in blood on GC-MS was developed using Clean Screen® DAU SPE cartridges and derivatization. BSTFA+10% TMCS was chosen as the derivatizing agent. A linear range of 0.015-0.20 mg/mL was established, and bias and imprecision tests met validation criteria. There was zero interference and carryover observed. Stability and recovery tests passed. Dilution integrity passed for 1:2, 1:10, and 1:50 dilutions. Matrix matching failed with liver and urine. The LOD was determined to be 0.001 mg/mL. Postmortem positive xylazine blood samples (n=14) were extracted and analyzed using this method to show it is fit for purpose. The ratios of central/peripheral range from 1.2:1-4:1. The peripheral concentration ranged from <0.015- 0.0642 mg/mL and central concentrations ranged from <0.015-0.172 mg/mL. Paired central and peripheral samples were analyzed with central blood sources yielding higher concentrations in 12 out of the 14 tested samples. The results indicate that postmortem redistribution (PMR) occurs for xylazine in a majority of cases.

Monitoring Drug Overdose Death in the United States, 1999-2023

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Introduction: Drug overdose death data from the National Vital Statistics System (NVSS) are used to monitor trends in deaths in the United States, track the characteristics of those who have died, and inform decisions about public health challenges. Provisional NVSS mortality data suggest that there were an estimated 107 thousand drug overdose deaths in 2023. In 2022, there were 107,941 drug overdose deaths, resulting in an age-adjusted death rate of 32.6 per 100,000 standard population. Overall, the age-adjusted rate of drug overdose deaths nearly quadrupled from 8.2 in 2002 to 32.6 in 2022. These data are gleaned from death certificates.

Objectives: This presentation will provide an overview of the mortality trends from the NVSS and will cover the process for collecting and compiling data from death certificates, including the role of the medical examiner, coroners and forensic toxicologists. In addition, the presentation will cover methods for coding causes of death according to the International Classification of Diseases 10th Revision and recent advances in data modernization such as examples of interoperability with medicolegal death investigation case management systems .

Methods: For the NVSS, drug overdose data are collected from information reported on death certificates filed in the 57 vital registration jurisdictions. NVSS functions with states maintaining autonomy in their vital registration operations and the federal government providing support, including coordinating functions and developing agreed upon standard specifications. Medical examiners and coroners play a central role in the system by determining cause and manner of death for sudden and unexpected deaths, including drug overdoses. Forensic toxicologists play a critical role by performing toxicological analysis when directed. Mortality data are coded according to the International Classification of Diseases which has codes for drugs and chemicals. In addition, data for specific drugs are coded according to the Drug Involved Mortality program codeveloped by CDC and the FDA.

Results: National, state and local vital statistical data are available in many forms to provide information to the public, public health and safety researchers, policy makers, media, and many others. Final annual data are released after all updates from the states and has been fully reviewed for completeness and quality. Final annual data are generally available within 1 year. Provisional drug overdose mortality data are available four months after the date of death which is useful for monitoring trends. This is closer to real time surveillance is due to advances in death certification processes. In 2022 about 70% of deaths were registered with 10 days of the date of death. Drug overdose death data continue to lag behind other causes due to the time to investigate the deaths.

Discussion: Statistics from the NVSS are used to monitor drug overdose death in the US. These data are from death certificates. Many factors related to death investigation and toxicological analysis may impact the measurement of drug overdose death rates. Of critical importance, death certifiers must recognize the role that the drugs played in the death, conduct an investigation, and importantly, work with forensic toxicologists to order and perform appropriate tests to determine the type of drugs present. The substances tested for and circumstances in which the tests are performed vary by jurisdiction. This presentation will discuss the implications of this variation in practices on the death statistics, and the important role of the forensic toxicologist.

Interpretation of prescription drug concentrations in opioid-related drug deaths. Should these drugs be included on the death certificate?

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Introduction: Opioid misuse is the leading cause of drug-toxicity deaths reported by the Office of the Chief Medical Examiner (OCME), Alberta, Canada.

A significant percentage of opioid-positive casework involves the concomitant detection of prescription drugs which can occasionally be present at concentrations which may be impactful or contributory to the overall assessment of drug-induced toxicity. However, the mere presence of a drug in a body fluid does not demonstrate impairment or intoxication and the subsequent naming/inclusion of a prescription drug on a death certificate impacts published data concentration ranges and prescription drug-related death statistics. It is therefore critical that medical examiners, pathologists and coroners carefully consider drug concentrations and the likelihood of that drug inducing or contributing to the overall toxicity when determining the cause of death.

Post mortem drug concentrations are typically higher than in-life ante-mortem drug concentrations due to a number of factors, most notably post mortem redistribution.

Objectives: Report mean and median concentration of prescription drugs in both femoral blood and vitreous humor in both the presence and absence of an opioid drug. Review cardiac : femoral drug concentration ratios.

Method: In all cases, post mortem femoral blood was collected from a visualized, ligated femoral vein during examination/autopsy, which was typically conducted within 48-72 hours of body-receipt at the morgue. Cardiac blood and vitreous fluid were collected at autopsy and examination/autopsy respectively. Drug screening using liquid chromatography time-of-flight (LC-TOF) and liquid chromatography tandem mass spectrometry (LC-MS/MS) targeted a range of commonly encountered controlled and abused drugs together with an expansive range of readily available, over-the-counter drugs, pharmaceutical compounds and commonly prescribed medications. Quantitative analysis for amitriptyline, citalopram and venlafaxine was undertaken using LC-MS/MS.

Results: The median femoral blood concentrations of Amitriptyline (0.345 mg/L), Citalopram (0.265 mg/L) and Venlafaxine (0.511 mg/L) in opioid positive casework are reported together with the median femoral blood concentrations of these drugs in cases where they were named in the cause of death: Amitriptyline (1.73 mg/L), Citalopram (4.16 mg/L), Venlafaxine (13.0 mg/L).

Vitreous drug concentrations are also reported in both opioid positive and 'named in cause of death' cases. Amitriptyline (0.078 and 0.62 mg/L), Citalopram (0.337 and 2.68 mg/L) and Venlafaxine (0.679 and 11.8 mg/L) respectively.

The Cardiac to Femoral blood concentration ratio was calculated for each drug together with the 95th percentile values, as detailed here: Amitriptyline 11.5, Citalopram 2.45 and Venlafaxine 4.80.

Discussion: By using known opioid-related death cases, threshold concentrations for pharmaceutical drug-related toxicity can be proposed in both femoral blood and vitreous humor, based on the fact that the presence of the pharmaceutical drugs, in these cases, was considered 'incidental' and deemed non-contributory to the cause of death.

Application of cardiac to femoral drug concentration ranges allows for estimation of a 'femoral blood equivalent concentration' in the absence of suitable samples. This is a useful tool for interpretation considering that cardiac blood is likely to be more affected by post mortem redistribution than ligated femoral blood.

Vitreous humor was chosen as an alternate specimen/matrices as it is relatively easy to sample/collect and this specimen is protected against contamination, putrefaction and degradation by microorganisms.

Toxicology Findings in Train-Related Pedestrian Fatalities in Harris County, Texas

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Introduction: Many studies have evaluated toxicology results of pedestrians involved in motor vehicle fatalities; however, fewer have assessed pedestrians struck by trains. Federal data indicate approximately half of train-related pedestrian fatalities involve alcohol or drugs but do not specify which drugs were identified. While cause of death (COD) in train-related cases is usually clear, manner of death (MOD) determinations can be challenging and may be informed by toxicology results.

Objectives: To evaluate toxicology findings in train-related pedestrian fatalities in the City of Houston and Harris County, Texas from 2019-2023.

Methods: The agency's information management systems were queried for train-related pedestrian fatalities from 2019-2023. ed demographics, case history, COD, MOD, and toxicology findings were evaluated. In general, alcohol and/or drug testing was performed on blood at the forensic pathologist's request. Alcohol testing included ethanol, methanol, isopropanol and acetone, with a 0.010 g/100 mL reporting limit. Drug screening was primarily performed by immunoassay and confirmations were performed by chromatography coupled with mass spectrometry.

Results: Medicolegal death investigations were performed for 37 train-related pedestrian fatalities. Most decedents were male (83.8%) and White (37.8%), with an average age of 45.8 years (range 17-79 years). Before impact, decedents were known to be laying (51.4%), standing (10.8%), walking (18.9%), kneeling (2.7%) or sitting (2.7%) on the tracks; circumstances were unknown in 13.5%. COD for all cases was related to blunt force injury. MOD was classified as suicide (43.2%), accident (51.4%) and undetermined (5.4%).

Toxicology testing was requested in 81% of cases; reasons for no testing included delayed death due to hospitalization and no pathologist request for testing. Of the 31 cases in which alcohol was tested, 12 (38.7%) were positive, with ethanol concentrations averaging 0.214 g/100 mL (range 0.016 – 0.460 g/100 mL). Of the 26 cases receiving drug testing, 12 contained one or more drug. Cocaine (n=5), methamphetamine (n=5), PCP (n=2), fentanyl (n=1), benzodiazepines (n=2), and antidepressants (n=1) were detected. Ethanol and drugs were combined in two cases. No ethanol or drugs were detected in nine cases (24.3%).

Ethanol and/or drugs were present in over half of the cases where the decedent was laying on (68.4%), walking on (57.1%), or seated on (100%) the track, or when positioning was unknown (60%). Ethanol and/or drugs were more likely to be present in cases classified as accident (73.7%) than suicide (43.8%) or undetermined (50.0%).

Discussion: In train-involved pedestrian fatalities, COD is often evident, but there may be questions regarding MOD. Toxicology findings may provide insight on the decedent's mental or physical faculties prior to death. Our case results are similar to federal data, in which more than half of fatalities included alcohol and/or drugs. The prevalence of alcohol and or drugs in our cases, however, may be underestimated, as many cases either did not receive testing or received limited toxicology testing that did not include psychiatric drugs, such as anti-depressants.

Similar to federal data, ethanol was most prevalent finding and was more often found in cases classified as accidents than as suicides. Most ethanol concentrations were consistent with psychomotor impairment and could explain terminal actions, such as laying on or walking on tracks. Federal data does not specify which drugs were present in fatalities. In our cases, central nervous system stimulants were most prevalent. The potential for increased risk-taking behaviors or poor judgement from stimulant intoxication cannot be ruled out; that would need to be supported by eye-witness account or video surveillance. Interestingly, no cases involved narcotic analgesics and few involved other central nervous system depressants, despite the high prevalence of these drug classes in other medicolegal death investigations.

P1

An Update to Human Red Blood Cell Acetylcholinesterase Activity Reference Ranges for Acute Organophosphate Poisoning Detection

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Introduction: Despite their highly valued effectiveness in agricultural pest control, many organophosphates are dangerous anti-cholinesterase agents. Exposure to even a tiny amount can cause serious adverse conditions. Acute organophosphate poisoning can rapidly deteriorate to severe respiratory failure, and may eventually lead to death. As a result, organophosphates have historically been utilized as poisons for nefarious purposes. For nearly a century, depression in human red blood cell acetylcholinesterase (RBC-AChE) activity has been recognized as a valuable biomarker for exposure to cholinesterase inhibiting organophosphates. Ideally, the individual RBC-AChE activity baseline should be used due to intra- and interindividual RBC-AChE activity variations. However, individual RBC-AChE activity baselines for most people are unknown. Therefore, an accurate population-based human RBC-AChE activity reference range has become critical for reliable early organophosphate poisoning screening. The current population-based adult human RBC-AChE activity reference range used by the U.S. federal government is 0.63-0.89 ΔpH/hour regardless of biological sex. It was adopted over 30 years ago and no updates since then.

Objectives: The purpose of this presentation is to describe an updated population-based adult human RBC-AChE activity reference range for rapid, economical, and reliable forensic toxicology screening for organophosphates and other cholinesterase inhibiting substances that cause acute poisoning.

Methods: The Cholinesterase Monitoring Program (CMP) is a federal government operated nation-wide testing network. It is consisted of the Cholinesterase Reference Laboratory (CRL) located at San Antonio, TX and multiple geographically dispersed satellite testing facilities. The CMP has utilized a time-modified Michel ΔpH testing method and maintained a database of a uniquely large amount of human adult RBC-AChE activity testing results under the Defense Public Health Centers. We conducted a retrospective analysis of the testing results collected by the CMP between January 2021 and December 2022 from 4,818 adult subjects in the age range of 18 to 80. Based upon the comparison of their testing values to their established individual RBC-AChE activity baselines, none of the subjects were considered having exposure to cholinesterase inhibiting organophosphates. We systematically investigated potential impacts of biological sex, continuous age, age group, and their combinations on interindividual RBC-AChE activity variations. To our best knowledge, our study is the first update on this topic in the past 15 years.

Results: Mean, median, and range of testing results for males, females, and the combined were calculated and compared to previously published data. When biological sex was the only factor considered, no significant difference (P=0.7238) was observed in the testing values between males (mean, median, interquartile range = 0.76, 0.76, 0.71-0.80 ΔpH/hour, respectively) and females (mean, median, interquartile range = 0.76, 0.76, 0.71-0.81 ΔpH/hour, respectively). An important new finding is that with age progression, male testing values exhibited a consistent upward trend, while females did not show any clear patterns. Linear regression analysis of the data revealed that biological sex, age, and age group affected testing values either as independent variables or with their combinations and interactions. However, more factors need to be included to achieve better testing value prediction models.

Conclusion/Discussion: Considering the difference in the mean testing values between the male age groups 18-29 and ≥60 was more than 8% of the mean value in the male age group of 18-29, we now recommend the forensic toxicology community to adopt a new set of age group specific RBC-AChE activity reference ranges for males (0.68-0.80, 0.69-0.81, 0.70-0.83, 0.71-0.84, and 0.73-0.87 ΔpH/hour for 18-29, 30-39, 40-49, 50-59, and ≥60 years old, respectively) while keeping the current federal RBC-AChE activity reference range for females.

P2

Detection of Xylazine in Whole Blood Samples Using High-Resolution Mass Spectrometry

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Introduction: Poly-drug intake, such as the addition of xylazine to synthetic opioids, significantly increases the risk of overdose. Individuals using illicit drugs might not be aware of the presence of non-opioid substances, such as xylazine, in drug products. Given the concerns about the role of xylazine in opioid overdoses, it is essential to employ a comprehensive drug screening approach. SWATH DIA is a valuable tool for this purpose as it collects MS/MS fragment data on all features present in a sample. This untargeted screening approach provides information to aid in identifying and quantifying known and emerging drugs and adulterants in toxicology samples.

Objective: The goal of this study was to describe a non-target acquisition method for the simultaneous screening and quantitation of the emerging drug xylazine in authentic human whole blood samples.

Methods: Authentic human whole blood samples were aliquoted (0.5 mL), pretreated with 3 mL of phosphate buffer (pH 6) and centrifuged at 3000 rpm for 10 minutes. The samples were loaded onto a solid phase extraction (SPE) cartridge consisting of a C8 and ion exchange phase (benzenesulfonic acid) bonded to the same particle. Cartridges were conditioned with methanol and water, followed by equilibration with 1 mL of phosphate buffer. After sample loading, the cartridge was washed with water, followed by 0.1M HCl, then methanol, before drying under vacuum. The analytes were eluted using 2 aliquots of 1.5 mL of 78:20:2 dichloromethane/isopropyl alcohol/ammonium hydroxide. Finally, 100 µL of 90:10 methanol/HCl solution was added, and the sample was dried under a nitrogen stream and reconstituted to 200 µL in 95:5 water/acetonitrile.

The injected sample volume was 10 µL. Analytical separation was performed using a Phenomenex Kinetex Core-shell C18 column (50 mm x 3 mm, 2.6µm). Mobile phase A consisted of water with 10mM ammonium formate at pH 3 with formic acid, while mobile phase B was 50:50 acetonitrile/methanol. MS and MS/MS data were collected using SWATH DIA on the SCIEX X500R QTOF system with SCIEX OS Software 3.3, each SWATH DIA scan beginning with a TOF MS experiment.

Results: A calibration curve containing a panel of 10 drugs, including xylazine, at concentrations ranging from 1 to 1000 ng/mL, was used for the quantitation of unknown whole blood samples. The lower limit of quantitation (LLOQ) was required to have an accuracy within ± 20% and a signal-to-noise ratio (S/N) greater than 10. For all 10 compounds, the LLOQ was successfully established at 1 ng/mL, which is sufficient for forensic screening methods.

Discussion: In our screening process, a total of seven whole blood samples known to contain fentanyl were analyzed. Notably, two of these samples contained xylazine at concentrations above the LLOQ, measuring 7.4 ng/mL and 8.3 ng/mL. Additionally, three samples exhibited detectable levels of xylazine but were found to be below the LLOQ. It is worth highlighting that none of the seven samples contained solely fentanyl but exhibited the presence of 2 to 3 additional drugs and/or metabolites from the panel. This underscores the complexity and potential poly-drug use associated with the source of these samples, demonstrating the importance of comprehensive screening and quantitation techniques in such analytical contexts. Given that the data was acquired through a non-targeted SWATH DIA approach, the processing method developed in this study for the targeted screening of specific compounds can be readily adapted to identify unknown compounds.

P3

Detection of Nitazenes in Vape Juice

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Introduction: The continuous emergence of novel synthetic opioids (NSOs) on the recreational drug market presents an additional challenge for drug tracking agencies and laboratories. In recent years, a class of synthetic opioids originally developed in the 1950s as analgesics, known as nitazenes, has been detected in the illicit drug supply and implicated in overdose mortality. Nitazenes vary greatly in potency and purity, and thus require only a small amount to cause acute intoxications. Their recent detection in illicit vape juice, resulting in accidental and fatal drug overdoses, poses a major challenge for public health officials.

Objective: This study aimed to analyze a collection of vape juice products purchased from vaping stores and screened for a panel of 15 nitazenes. A quantitative liquid chromatography-tandem mass spectrometry (LC-MS/MS) method was developed to accurately detect low levels of nitazenes in the vape juice products analyzed.

Methods: Three vape juice samples were purchased from vaping stores. The samples were diluted 1,000 times in methanol and directly injected for LC-MS/MS analysis. The LC analysis was performed using a Shimadzu LC-40 at a flow rate of 0.6 mL/min using a Phenomenex Kinetex 2.6 µm F5 100 Å 50x2.1 mm column (Phenomenex, Torrance, CA). The injected sample volume was 10 µL. Mobile phases A and B were 10 mM ammonium formate in Optima grade water and Optima grade methanol with 0.02% formic acid, respectively. Samples were then injected into the QTRAP 4500 system, and MS/MS data were acquired using positive electrospray ionization. A single acquisition method consisting of 34 MRMs (30 for the analytes and 4 for the internal standards) was created and used to analyze the samples.

Results: The three vape juice products diluted 1,000 times in methanol and spiked with the 15 nitazenes were injected at six concentration levels ranging from 0.5 pg/mL to 100 ng/mL for all anyaltes to assess the quantitative performance of the developed method. Excellent linearity was observed across the concentration ranges analyzed, with R^2 values greater than 0.99 for all the nitazenes targeted in this panel. The method used demonstrated excellent recoveries (% recoveries >80%). In addition, the method showed low variability between injections with % CV <10 for all the analytes included in the panel, demonstrating the high level of reproducibility.

Discussion: A comprehensive workflow for the detection of 15 nitazenes in vape juice was developed. A fast and simple preparation method combined with a robust acquisition method enabled the accurate quantitation of 15 nitazene compounds. The optimized LC conditions resulted in the separation of the drugs in a 15.5-minute runtime, while the use of the MRM workflow enabled sensitive and accurate quantification of the 15 nitazene compounds across six calibration levels. In addition, the robustness of the developed workflow enabled reproducible and sensitive quantitation of the analytes, making this method suitable for screening vape juice products for the presence of potent and highly toxic nitazene compounds.

P6

Δ-8- and Δ-9-THC-Carboxy Metabolites in Urine Drug Testing Specimens at CRL from April 2023 to January 2024

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Introduction: Since gaining popularity in 2020, Δ8-THC has had a direct impact on the drug testing industry, creating potential analytical interference as well as negative confirmation results in tests with reporting criteria specific to Δ9-THC metabolite. As more states have passed legalization of Δ9-THC, the widespread usage of Δ8-THC has perpetuated as a means to pass drug tests when Δ9-THC use is still restricted.

Objective: Evaluate the presence of Δ8-COOH-THC in nonregulated urine drug testing samples being confirmed for Δ9-COOH-THC, and examine the prevalence of Δ8-COOH-THC among samples categorized by reason for test.

Methods: From April 2023 through January 2024, a total of 108,131 urine specimens screened positive for cannabinoids by immunoassay and were analyzed by LC-MS/MS for confirmation. The confirmation assay, which had been optimized for separation of Δ8- and Δ9-COOH-THC, was validated in accordance with National Laboratory Certification Program guidelines, including interference studies involving numerous cannabinoids and other drugs. In addition to separating Δ8- and Δ9-COOH-THC, ions were collected and quantitative results were processed for each analyte in nonregulated specimens.

Results: All specimens were de-identified and detached from client affiliation, with results reporting pursuant to client account and drug testing policy. The overall confirmation positivity rate for Δ9-COOH-THC was 82% with a 15 ng/mL cutoff, a significant decline from the almost 100% confirmation rates in years prior to 2020. Samples screening positive but reporting negative with a 15 ng/mL cutoff included 6.2% that contained only Δ8-COOH-THC; 4.5% with Δ9-COOH-THC at a concentration greater than the assay LOQ of 3 ng/mL but less than 15 ng/mL; 4.8% that were positive for Δ8-COOH-THC at concentrations greater than the 15 ng/mL cutoff but had detectable levels of Δ9-COOH-THC less than 15 ng/mL; 1.4% with both Δ8- and Δ9-COOH-THC at detectable levels less than 15 ng/mL; and 1.1% of samples having both Δ8- and Δ9-THC metabolites less than 3 ng/mL. Δ8-COOH-THC was detected in 56.0% of the samples that screened positive, and Δ8- and Δ9-COOH-THC were both detected in 49.8% of the positive screens. For samples with detectable levels of Δ8-COOH-THC, 8.5% had concentrations greater than the upper limit of assay linearity of 500 ng/mL, with the highest concentration reaching almost 70,000 ng/mL.

When categorized by reason for test, Δ8-COOH-THC was detected in more than 60% of Post-Accident, Reasonable Cause, and Follow-Up tests. However, Post-Accident and Reasonable Cause tests had lower concentrations of Δ8-THC metabolite overall, with Δ8-COOH-THC comprising less than 5% of the total carboxy-THC in more than 35% of screened positive samples in these categories. Among reason for test groups with sample populations exceeding 1,000, the highest percentages of samples with only Δ8-COOH-THC, as well as samples reporting negative for Δ9-COOH-THC having Δ8-COOH-THC concentrations greater than 15 ng/mL, were attributed to Follow-Up tests. Δ8-COOH-THC made up 75% or more of the carboxy-THC metabolite concentrations for more than 20% of Follow-Up tests with detectable Δ8-THC metabolite.

Discussion: The presence of Δ8-COOH-THC in urine drug testing samples continues to reveal a threat to public safety that is going largely unaddressed. Almost 10% of samples screening positive for cannabinoids report negative for Δ9-COOH-THC concentrations less than the 15 ng/mL cutoff, but in fact have Δ8-COOH-THC concentrations greater than 15 ng/mL. Because Δ8-THC is intoxicating, inexpensive, and ambiguously legal, it will likely continue in popularity until corporate and federal policy include Δ8-THC testing in addition to Δ9-THC.

P7

Trends in Fentanyl Analysis in Workplace Urine Drug Testing at CRL

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Introduction: Fentanyl began its dramatic impact on the opioid epidemic around 2013, and since 2021 has turned this public health emergency into a polysubstance overdose crisis. Combined with other drugs, fentanyl boosts potency and causes fatalities at lower than expected doses. The United States Center for Disease Control (CDC) and Drug Enforcement Administration (DEA) have reported that fentanyl has contributed to an estimated 70% of more than 300,000 overdose deaths in the US over the last three years. CRL began providing urine fentanyl analysis to clients in 2006, but increasing fentanyl abuse has caused more employers to add fentanyl to their standard workplace testing panels.

Objective: Explore trends in fentanyl analysis in workplace urine drug testing through examining positivity rates, reasons for test, and concurrence of fentanyl positive results with other drugs of abuse.

Methods: CRL tests for fentanyl and its metabolite norfentanyl in urine through enzyme immunoassay (EIA) screening with confirmation of all presumptive positives by HPLC-MS/MS. Cutoff concentrations for both screening and confirmation are variable by client, with screening cutoffs most commonly at 1.0 ng/mL, followed by 0.5 ng/mL, and confirmation cutoffs most commonly 0.5 ng/mL, followed by 1.0 ng/mL. For this study, demographic information and reported results for workplace urine drug testing samples analyzed at CRL from January 2022 to January 2024 were reviewed; specimens tested through professional monitoring programs, parole and probation groups, and rehabilitation services were omitted. Either fentanyl or norfentanyl concentrations greater than the cutoff were considered a positive reported result.

Results: For almost 5,000 workplace urine drug testing specimens screening positive by immunoassay, the overall confirmation rate was 87%. Reported positives were comprised of 41% of samples testing greater than cutoff for both fentanyl and norfentanyl, 33% testing positive for fentanyl and less than cutoff for norfentanyl, and 13% testing below cutoff for fentanyl but positive for norfentanyl. Median positive values for fentanyl and norfentanyl were 12.6 ng/mL and 40.0 ng/mL, respectively. Mean positive results were 257.5 ng/mL for fentanyl and 1025.4 ng/mL for norfentanyl. Maximum concentrations were 36,199 ng/mL for fentanyl and 52,595 ng/mL for norfentanyl.

Random drug screens produced 57.5% of fentanyl positives, while the reasons for test “other” and “reasonable cause” combined for almost 25% of reported positives. Pre-employment tests accounted for only 5.5% of fentanyl-positive samples.

A total of 64.8% of fentanyl positives were polydrug positives, and 21.2% of those samples were positive for 3 or more drugs in addition to fentanyl. The drug most commonly found in combination with fentanyl was THC, appearing in 22.6% of fentanyl positives. This was followed by amphetamine and methamphetamine, which were present in 20.4% and 19.9% of fentanyl-positive samples. Only 12% of fentanyl polydrug positives involved an opiate, but of those samples, almost 60% contained 2 or more opiates.

Discussion: The typical detection window for fentanyl and norfentanyl in urine is 2-4 days, but can extend past 7 days depending on dosage, frequency of use, and testing methodology. The high confirmation rate for fentanyl in workplace urine drug testing at CRL indicates the importance of this testing for workplace safety; with the majority of workplace urine drug tests being conducted as pre-employment screens, the number of fentanyl positives generated from random drug tests was alarming. Trends in concurrent drug use with fentanyl were supported by the numbers of polydrug positives observed, especially the combinations with stimulants like amphetamine and methamphetamine. Fentanyl-opiate combination positives were lower than expected, considering the prevalence of fentanyl-adulterated heroin and counterfeit prescription opioid pills.

Conclusion: This study indicates that workplace urine fentanyl testing results reflect current patterns of concern in the opioid epidemic. CRL offers 1600 testing panels that include fentanyl, providing various cutoffs and accompanying analytes. With no foreseeable decline in fentanyl distribution and abuse, it is essential for employers to recognize the importance of fentanyl testing to workplace safety.

P8

CRL Testing Experience for Fentanyl in Oral Fluid

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Introduction: The evolution of fentanyl from effective analgesic to national abuse crisis has been a well-documented tragedy over the last decade. Fentanyl is used in pure form, combination drug cocktails, counterfeit prescription pills, and in drugs covertly spiked to increase potency. With strength 100-fold greater than morphine and 50-fold more powerful than heroin, fentanyl has contributed to well over 200,000 overdose deaths in the United States since 2021.

CRL began testing for fentanyl and its metabolite norfentanyl in oral fluid in 2018. As opioid abuse has grown to epidemic proportions, employers have added fentanyl to routine drug testing panels for increased workplace safety.

Objectives: Evaluate the CRL workplace drug testing experience for fentanyl in oral fluid specimens, from screening to confirmation, including reasons for test and polydrug positive results.

Methods: A retrospective analysis was performed on reported results and demographic data for oral fluid workplace drug testing samples analyzed at CRL from January 2022 to 2024. Sample collection was performed using OraSure Intercept devices, which provide a 1.2 mL total specimen volume through the combination of 400 µL of oral fluid collected onto the device pad and 800 µL of buffer in the device tube. Because of this combined specimen/buffer composite, oral fluid confirmation results are reported as diluted values and results require multiplication by 3 to attain neat values.

Oral fluid specimens requiring fentanyl testing are prepared for screening by dilution with aqueous mobile phase, while the confirmation assay requires a liquid-liquid extraction preparation. Samples are screened and confirmed by separate LC-MS/MS analytical methods using Shimadzu Nexera UHPLC systems and API6500+ Mass Spectrometers.

Results: Using a 1.0 ng/mL fentanyl cutoff concentration for both screening and confirmation, 97.9% of samples screening positive were confirmed. Median positive fentanyl and norfentanyl concentrations were 19.7 ng/mL and 4.5 ng/mL, or 59.1 and 13.5 ng/mL as neat values. Mean positive fentanyl and norfentanyl levels were 84.6 and 14.6 ng/mL, or 253.8 and 42.8 ng/mL neat. Maximum fentanyl concentrations reached the upper level of assay linearity at 2,000 ng/mL, or 6,000 ng/mL neat; maximum norfentanyl concentration was 250 ng/mL, or 750 ng/mL neat value.

Categorized by reason for test indicated on sample requisitions, 43.2% of oral fluid fentanyl positives were a result of workplace “Random” tests. While pre-employment drug screens account for more than 50% of testing, this category attributed to a mere 4.2% of fentanyl positives.

More than 70% of oral fluid fentanyl positives also confirmed positive for at least one other drug. Methadone was most prevalent in these polydrug combinations, appearing in 29.2% of positive fentanyl samples. Stimulants such as methamphetamine and cocaine were also frequently found in fentanyl positives; fentanyl and methamphetamine/amphetamine combinations made up 26.7% of the concurrent positives, while fentanyl and cocaine combinations occurred in 24.4% of concurrent positives.

Discussion: A common misconception is that fentanyl and norfentanyl are unable to be detected in oral fluid, but the testing experience at CRL proves quite the opposite. Specimens from professional monitoring programs, rehabilitation, and probation parole groups were eliminated from the data pool; though requests for fentanyl in oral fluid workplace drug testing were relatively low, the positivity rate was 3-5%. The number of polydrug positives including fentanyl was unsurprising, with cases of stimulant-fentanyl combinations in sync with current trends in overdoses; it was unexpected, however, that among fentanyl polydrug positives, fewer than 15% included opiates. The prevalence of fentanyl in random drug tests suggests high potential for workplace impairment, as detection period of fentanyl in oral fluid is 24-48 hours depending on dosage and frequency of use.

Conclusion: Employers must be vigilant regarding the pervasiveness of fentanyl in the workplace, and oral fluid testing allows for non-invasive collection with a detection period relevant to present-time impairment. Analysis of oral fluid samples for fentanyl at CRL offers employers an important tool to help maintain a safe work environment.

P9

Quantitation of an Oral Fluid Drug Panel Including THC Using High Resolution Accurate-Mass (HRAM) Orbitrap Mass Spectrometry

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Introduction: As labs move towards oral fluid for ease of collection and roadside testing, it is important to be able to test for a wide range of analytes and achieve required sensitivity. With the SAMHSA guidelines providing LOQ levels, the extraction protocol and instrumentation need to be sensitive enough to accomplish these cut-offs. Including tetrahydrocannabinol (THC) into the assay provides challenges in the extraction as most drugs of abuse are basic and THC is neutral. This extraction workflow, which extracts THC alongside other drugs of abuse, coupled with the Orbitrap™ mass spectrometer generates high-resolution accurate mass data that offers improved sensitivity, selectivity, and accuracy for detection and quantitation of drugs of abuse in oral fluid.

Objectives: To develop a HRAM LCMS method for quantitative analysis of 31 drugs of abuse in oral fluid with a complete sample preparation workflow that satisfies cutoff levels presented by the National Safety Council Tier 1 Drugs and SAMHSA.

Methods: Nine calibration levels (ranging from 0.5 to 1000 ng/mL) were made by spiking stock solution of the 31 target analytes into human oral fluid. Spiked samples were diluted with a Quantisal™ preserving buffer and spiked with their corresponding internal standard. 500 µL of each sample were then extracted using DPX INTip™ SCX/WAX SPE. Drug analytes were separated with on a Thermo Scientific™ Accucore™ Biphenyl column connected to a Thermo Scientific™ Vanquish™ Horizon UHPLC system using a fast 7-minute method. Data was acquired on the Thermo Scientific™ Orbitrap™ Exploris™ 120 mass spectrometer using data dependent MS2 mode (ddMS2) with an inclusion list for the 31 target drugs. Thermo Scientific™ TraceFinder™ 5.2 software was used for data acquisition and processing.

Results: Limits of quantitation (LOQ) determined for the 31 drugs were all below the new SAMHSA guidelines cutoffs and linearity was achieved from as low as 0.5 ng/mL to a ULOL of 1,000 ng/mL. THC delta-9 achieved an LOQ of 1 ng/mL. All drugs were confirmed with mass accuracy of less than 5ppm, retention times, and library matching. This study was also able to overcome some of the issues commonly associated with THC including “stickiness” of the drug to consumables and its susceptibility to being suppressed by oral fluid collection device buffers.

Discussion: This fast and quantitative method was developed around 31 drugs of abuse specified by SAMHSA and the National Safety Council. A complete workflow was presented that involved sample preparation using DPX INTip SPE. Linearity was achieved from LOQ’s as low as 0.5 ng/mL up to 1,000 ng/mL which exemplifies the sensitivity of these instruments and extraction procedure. This fast and sensitive method for oral fluid testing sufficiently passes the SAMHSA guidelines.

P10

A Fully Validated LC-QTOF-MS/MS Screening Workflow for the Analysis of Drugs in Oral Fluid

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Introduction: To date, very few police agencies are utilizing oral fluid as a viable testing matrix for testing DUID cases. Oral fluid is considered a viable alternative matrix since drug presence in oral fluid reflects that of free substances in the blood. Oral fluid, in contrast to blood collection, is considered non-invasive. Currently, most drug testing involves screening samples by immunoassay-based testing kits, enzyme-linked immunosorbent assays (ELISA) being the most common. The current drug testing environment is seeing a greater expanse of new psychoactive substances (NPS). The ability to screen specimens for a wide range of drugs, including NPS would be a significant improvement on currently used techniques.

Objectives: The objective is to develop a simple liquid-liquid extraction procedure (LLE) with analysis by liquid chromatography quadrupole time of flight tandem mass spectrometry (LC-QTOF-MS/MS) for drugs in oral fluid collected with the Quantisal™ device has been developed. The decision point cut-off concentrations were at or below those recommended by the National Safety Council's Alcohol, Drugs, and Impairment Division for toxicological investigation of driving under the influence of drugs cases (DUID).

Methods: Briefly, Quantisal (1mL) was mixed with isopropanol, ethyl acetate / hexane, 2% ammonium hydroxide in ethyl acetate, centrifuged, and decanted; followed by a second liquid-liquid process which extracted all the drugs in a single aliquot. A gradient liquid chromatography program using 0.1% formic acid in water and 0.1% formic acid in methanol was used and the runtime was 7.5 minutes. The tuning parameters for operation of the Q-TOF are critical: both QTOF and MS/MS were tuned in SureMass utilizing the setting of 2 GHz for extended dynamic range with mass collection up to 1700 m/z. The slicer was set at 1 to achieve high sensitivity by passing ions through a wider portion. In the system, reference ions are collected for m/z 121 and m/z 922, while the instrument is run in "All Ions" mode which collects all masses between 40 and 1000 m/z, and all subsequent fragments generated at collision energies 0, 10, 20, and 40V. Instrument settings were optimized with the nozzle voltage being the most important. The voltage varied across the run: at 0.3 min it was set at 100V to optimize opiate response; at 7.6 minutes it was increased to 2000V to promote Δ9-THC detection.

The supporting mass spectral-based screening library was adapted from commercially available databases and included Tier 1 and Tier 2 recommended compounds. Further, the additional inclusion of novel psychoactive substances and synthetic cannabinoids was based on the CFSRE's quarterly publications of 2023. Metabolites from those publications were not included in this method since, with some exceptions, parent drugs are the dominant compounds in oral fluid.

Discussion: The method was fully validated using ANSI/ASB 036 Standard Practices for Method Validation in Forensic Toxicology as guidance. Interference studies, limit of detection, precision at and around the decision points, ionization suppression/enhancement, and processed sample stability up to 96 hours were completed for each drug in the library database. All library database entries were compared against known certified reference materials. While ion suppression or enhancement of the analytes varied greatly, the decision point did not seem affected and internal standards that mimicked similar responses were chosen for each analyte. The method was applied to the 2023 proficiency program samples, routine samples received into the laboratory, and blind samples screened against the library search engine. The optimization of specific tune characteristics and instrument settings allows the user to meet or exceed recommended screening limits for drugs in Quantisal™ collected oral fluid samples without the need for immunoassay testing.

P11

Comparison of Return on Investment of a LC-QTOF Screening Method and an ELISA Screening Method

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Introduction: Toxicology laboratories require efficient, accurate, and affordable methods for screening biological samples for drugs of abuse. Historically, immunosorbent assay (IA) remains one of the most commonly utilized methods due to its fast analysis time for multiple drug classes across a large batch of samples.

The Orange County Crime Lab (OCCL) has recently introduced a screening method into its workflow using a liquid chromatograph quadrupole time of flight (LC-QTOF). The LC-QTOF is known for its high specificity, large scope, and relatively short analysis time per sample. Screening exclusively with LC-QTOF remains less common than IA due to the perceived monetary and time investment needed from laboratories to transition from IA to LC-QTOF.

Objectives: Compare the return on investment of screening with IA versus LC-QTOF. Comparison will include the initial cost, the operating cost, and analytical outputs of both methods.

Methods: OCCL cases are screened using acetonitrile protein precipitation on LC-QTOF and using a buffer dilution on IA. A comparison was done of reported results where LC-QTOF findings that are not covered by IA assays, including cross-reactivity, were identified. An eleven-assay IA panel was used for the comparison including amphetamine, barbiturates, benzodiazepines, cannabinoids, cocaine, fentanyl, methamphetamine, opiates, oxycodone, PCP, and zolpidem. Cross-reactivity information was taken from the product inserts from Immunalysis. 1000 antemortem (AM) cases and 1020 postmortem (PM) cases were evaluated.

Actual costs from the OCCL were collected to determine initial capital investments (one-time costs), and a “per sample” cost per analysis. Costs include extraction materials, instrument costs, and maintenance. Other considerations, such as analyst time, required training, and data storage, will also be discussed.

Results: For the AM cases, 20% of the drugs reported would not have been detected without an LC-QTOF. Most of the drugs in these cases were SSRIs or were found in conjunction with other impairing drugs such as ethanol. For the PM cases, 37% of the drugs reported would not have been found without an LC-QTOF. These drugs presented a greater variety of drug classes than those of the AM cases, however, naloxone represented the largest portion of the 37%.

Initial capital investment for the LC-QTOF screening method totaled approximately $389,621 while the initial capital investment for the IA screening method totaled approximately $325,014. Per sample costs begin at $9 for LC-QTOF and $15 for IA including consumables and maintenance contracts and increase when considering analyst time, data storage, etc.

Discussion: There were minimal differences found for driving-under-the-influence (DUI) cases between the IA and LC-QTOF workflows. For postmortem samples, however, LC-QTOF may be more impactful. The ability to detect more drugs that do not cross-react on IA is invaluable in providing additional information to the toxicologist during interpretation.

Additionally, the sample costs for LC-QTOF were almost 50% less than those of IA. Screening with LC-QTOF is therefore more economical over a long-term timeframe. Laboratories that analyze only DUI cases may be sufficiently covered by IA-based workflows, but at a greater cost per sample than if LC-QTOF was used.

While IA can screen for a large variety of drug classes and cross-react with many other drugs, LC-QTOF can provide more information at a more economical cost per sample. While the benefits of greater scope and specificity cannot be entirely quantitated, they should be considered as to whether the initial capital and time investments of LC-QTOF are justified. Laboratories performing screening exclusively with IA should consider the analytical benefits and long-term savings of switching screening methods to LC-QTOF.

P12

Nitazene analog detection and involvement in drug overdose deaths — United States, 2019–2022

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Introduction: Nitazene analogs (or "nitazenes") are a subclass of synthetic opioids that have been linked to overdose outbreaks in North America. These substances have high binding affinity at the μ-opioid receptor. Naloxone can reverse nitazene overdoses because nitazenes are opioids; however, their potency might necessitate multiple doses. Nitazenes are present in the illegal drug supply, but the extent to which they are detected and involved in overdose fatalities has not been fully explored. People who use drugs may be unknowingly exposed to nitazenes, increasing their risk for overdose or adverse effects. Ongoing data on nitazene detection and involvement in overdose deaths is critical to inform the scope of forensic toxicology testing and ensure the timely detection and mitigation of nitazene-related overdose outbreaks.

Objectives: Describe quarterly trends in the number of overdose deaths with nitazenes detected or involved, overall and by specific analog, during 2019–2022. Other drugs commonly co-detected are also reported.

Methods: CDC's Overdose Data to Action (OD2A) program funded 47 states and Washington, DC to collect and report data on unintentional and undetermined intent overdose deaths to the State Unintentional Drug Overdose Reporting System (SUDORS) twice yearly (deaths during January–June and July–December). Funded jurisdictions ed data from death certificates and medical examiner/coroner reports, including complete postmortem toxicology results. Descriptive analyses of overdose deaths that occurred during July 2019–December 2022 were conducted, limited to 45 jurisdictions with toxicology reports available for ≥75% of overdose deaths in any 6-month reporting period during the study period. Analyses included the number of overdose deaths with nitazenes detected, the proportion of deaths where nitazenes contributed to death (i.e. "involved"), and the proportions of deaths with other drugs co-detected, such as illegally-made fentanyl (IMF) and novel benzodiazepines. Quarterly trends in overdose deaths are reported for the 25 jurisdictions with toxicology reports available for ≥75% of overdose deaths for all 6-month time periods during July 2019–December 2022.

Results: Nitazenes were detected in 615 overdose deaths across 36 jurisdictions during July 2019–December 2022. The most commonly detected analogs were metonitazene (n=353; 23 jurisdictions), isotonitazene (n=229; 22 jurisdictions), protonitazene (n=50; 16 jurisdictions) and *N*-pyrrolidino etonitazene (n=26; 9 jurisdictions). Nitazenes were involved in 83.6% of the deaths in which they were detected. IMF was detected in 77.6% of deaths in which nitazenes were detected; co-detection ranged from 46.2% (*N*-pyrrolidino etonitazene) to 88.4% (metonitazene). Novel benzodiazepines were detected in 26.0% of deaths in which nitazenes were detected. In the 25 jurisdictions with consistent data across the study period, isotonitazene was first detected in a fatal overdose in August 2019; detection rose until peaking in the first quarter of 2020. Protonitazene was first observed near the end of the study period, and deaths with protonitazene detected doubled from 7 to 17 between the 3rd and 4th quarters of 2022.

Discussion: An increasing variety of highly potent nitazenes were detected in small but persistent numbers of fatal overdoses reported to SUDORS during 2019—2022. The most frequently detected nitazenes varied over time with specific peaks and declines that may be related to U.S. Drug Enforcement Agency scheduling. IMF was co-detected with nitazenes in most overdose deaths, but about half of cases with more recently detected analogs (*N*-pyrrolidino etonitazene and protonitazene) did not have IMF detected. This may indicate a distinct nitazene analog product and requires monitoring. About one-quarter of deaths with nitazenes detected also had novel benzodiazepines detected, potentially indicating mixing or selling together of these rarer drugs. Because of the high potential for overdose associated with highly potent nitazenes, ensuring consistent routine postmortem toxicology testing for these substances is important for timely monitoring and response.

P13

Occurrence of Opioids in Forensic Casework in the State of Sergipe in Brazil (2019 - 2023)

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Introduction: Opioids are substances frequently reported in forensic casework. Opioid misuse is a global public health problem and has been on the rise in some countries. For example, in the United States, opioid-related deaths have increased between 2019 and 2021 [1]. However, in contrast, opioids have not been historically widely abused in Brazil.

Objectives: The goal of this study was to review opioid-related cases in the state of Sergipe, in the Northeast Region of Brazil.

Methods: Data from 1,733 forensic cases received by the Forensic Toxicology Laboratory of the Scientific Police of Sergipe between January of 2019 and August of 2023 were retrospectively reviewed. Non-personally identifiable demographic information (gender, age, and case circumstance) and toxicology results obtained via liquid chromatography-triple quadrupole mass spectrometry (LC-MS/MS) were collected. This study was determined to not involve human subjects, not requiring Institutional Review Board approval.

Results: Results are summarized in Table 1. Opioids were detected in samples collected from cases classified as follows: motor vehicle accidents, fatal intoxications, non-fatal intoxications, cause of death pending and others.

Table 1. Summary of opioid identification in forensic casework received by the Forensic Toxicology Laboratory of the Scientific Police of Sergipe from January of 2019 through August of 2023

		2019	2020	2021	2022	2023
Cases positive for opioids (% relative to all cases positives for drugs, medicines, and pesticides)		2 (5.4%)	9 (14.5%)	7 (8.9%)	29 (14.9%)	9 (8.2%)
Demographics	Gender	Male: 50% Female: 50%	Male: 67% Female: 33%	Male: 71% Female: 29%	Male: 79% Female: 21%	Male: 56% Female: 44%
	Age	45*	Mean: 31.2 years Median: 34 years	Mean: 31 years* Median: 31.5 years	Mean: 41.4 years Median: 37 years	Mean: 36.4 years Median: 38 years
Toxicological Results (opioids detected individually or in the presence of other opioids or classes of drugs)		Fentanyl (n = 1), tramadol (n = 1)	Fentanyl (n = 4), tramadol (n = 2), morphine (n = 2), acetyl norfentanyl (n = 1), hydromorphone (n = 1) and methadone (n = 1)	Fentanyl (n = 4), morphine (n = 2) and methadone (n = 1)	Fentanyl (n = 21), morphine (n = 4), methadone (n = 4), tramadol (n = 4), codeine (n = 1) and hydromorphone (n = 3)	Fentanyl (n = 7), morphine (n = 1), hydromorphone (n = 1) and methadone (n = 1)

*Data not available for one case

Discussion: The number of cases involving opioids was low compared to all cases positive for at least one drug, medicine, and pesticides. Some cases involving opioids were associated with administration by medical personnel. In the first two years of the pandemic (2020 – 2021), the increase in the number of cases positives for opioids (compared to 2019) could also be associated to the administration of fentanyl and midazolam in a hospital setting; the same combination was also observed in cases in 2022 (n = 9) and 2023 (n = 3). Although the statistical population is not significant to identify any trends and does not represent the whole country, this data suggests that the prevalence of opioids in forensic casework from 2019 through August of 2023 was limited, in contrast to other countries such as the United States, where opioids were involved in more than 75% of reported deaths by drug overdose in 2022 [1], and the European Union, where opioids were reported in 74% of overdose deaths in 2021 [2]. Geographical and cost factors may explain an easier access to other drugs, leading to a lower availability of opioids in the illicit drug supply in Brazil and therefore limited occurrence in casework in the period. Nevertheless, the presence of opioids due to legitimate medical use and the emergence of novel synthetic opioids (which may not be monitored yet) must also be considered when assessing potential/future trends.

References

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P14

Integration of Ultrashort-Chain Compounds into the Biomonitoring of Per- and Polyfluorinated Substances in Human Plasma and Serum

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Introduction: The selection of per- and polyfluoroalkyl substances (PFAS) for biomonitoring can vary depending on regional or population-specific concern. Commonly examined PFAS encompass a spectrum from short- to long-chain compounds (C4 – C10). The ultrashort-chain (USC) PFAS with carbon chain lengths of shorter than C4 have become a major concern due to their prevalence and high levels of occurrence in environmental aquatic systems. Numerous studies have observed a rapid escalation in environmental concentration of USC PFAS, raising the concern of elevated human exposure. Assessing USC PFAS levels in blood not only facilitates monitoring of human exposure but also provide a tool to investigate potential risks associated with such exposure.

Objectives: This study aimed to develop a simple and reliable workflow to simultaneously quantify C1 to C10 perfluoroalkyl carboxylic acids and perfluoroalkyl sulfonic acids, along with four alternative compounds, in human plasma and serum.

Methods: An LC method with 11 minutes of cycling time was developed to achieve optimal retention for USC PFAS under reversed-phase conditions. More importantly, the extensive retention of USC PFAS led to reduced matrix interference. Three batches of accuracy and precision analyses were conducted using a charcoal-stripped fetal bovine serum fortified with target PFAS at 0.4, 2, 10, and 30 ppt. A 100 µL aliquot of serum sample was mixed with both extracted and non-extracted internal standards, along with 200 µL of methanol containing 1.5% formic acid. After centrifugation, 5 µL of supernatant was injected onto a polar-embedded alkyl phase column (Ultra IBD, 100 x 2.1 mm) for chromatographic separation followed by MS/MS detection. Calibration standards were prepared in the range of 0.05 to 40 ppb in reverse osmosis water containing phosphate-buffered saline. The established method was employed to measure PFAS in NIST 1950 and 1957 standard reference human plasma and serum with known concentration of six or seven short-chain and long-chain PFAS.

Results: Employing quadratic regression (1/x weighted), all analytes could obtain proper linearities with r^2 >0.995 and deviations <20%. For accuracy and precision analysis, all analytes exhibited accuracy values within the range of 82.3 – 115% across all fortification levels. Satisfactory method precision was demonstrated with %RSD values ranging from 0.965 to 11.3%. All extracted internal standards had accuracy values within 20% of the nominal concentration. The analysis of PFAS in two NIST standard reference materials showed that the averaged experimental concentrations of most PFAS closely matched the reference concentrations, with deviations within 20%. These results demonstrated that the established method was suitable for accurate measurement of PFAS in both human plasma and serum.

Discussion: A simple and reliable workflow was established in this study to provide a unique solution for the integration of ultrashort-chain compounds into the measurement of PFAS in human plasma and serum. The reported method was rugged, accurate, and precise by implementing a polar-embedded column for chromatographic analysis. Most importantly, this solution can offer a valuable tool for gaining insights into human exposure to these emergent ultrashort-chain PFAS.

P15

Assessment of Δ9-THC and Δ9-THCCOOH Bias, Precision, and Ionization Suppression/Enhancement between Solid Tissue Homogenate and Supernatant by LC-MS/MS

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Introduction: LC-MS/MS assays are frequently utilized for screening and confirmatory purposes in the forensic toxicology laboratory. While these techniques are excellent for the targeted identification and quantitation of a wide variety of drug classes, validation and determining fit-for-purpose is a significant requirement for each method. In the United States, ANSI/ASB Standard 036 currently serves as a primary resource in forensic toxicology method validation, and mandates that laboratories evaluate critical performance characteristics to help ensure the production of forensically defensible results. Due to the variability of specimen quality frequently encountered in the discipline of postmortem toxicology, the State of Maryland Office of the Chief Medical Examiner Forensic Toxicology Laboratory routinely analyzes solid tissue specimens as part of the medicolegal death investigation process and evaluates liver as a representative solid tissue matrix during method validation. Authentic postmortem specimens were used to investigate the effects of analyzing solid tissue homogenate versus solid tissue supernatant on bias, precision, and ionization suppression/enhancement of Δ^9-THC and Δ^9-THCCOOH. Noticeable quantitative differences were observed in authentic postmortem solid tissue homogenate and supernatant specimens despite evaluating from identical tissue samplings. A brief discussion of the results is presented using a validated LC-MS/MS method for the confirmation and quantitation of Δ^9-THC and Δ^9-THCCOOH in postmortem casework.

Objectives: To evaluate how the differences in bias, precision, and ionization suppression/enhancement in solid tissue homogenate and supernatant may affect drug quantitation and the laboratory's overall method validation procedures.

Methods: Solid tissue specimens were prepared as a 1 to 5 dilution by adding 20 g ultrapure water to 5 g of tissue (25 g total) which was then mechanically homogenized. One milliliter of specimen was then sampled directly (homogenate) or centrifuged at approximately 2230 x *g* for 10 min prior to sampling (supernatant). Δ^9-THC and Δ^9-THCCOOH were then isolated from case specimens by means of a liquid-liquid extraction scheme. In short, the solid tissue homogenate or supernatant specimen was mixed with ultrapure water and 10% acetic acid. A total of 6.0 mL of a 9:1 mixture of hexane:ethyl acetate was then added to facilitate the extraction. The resultant solvent layer was evaporated to dryness, reconstituted in mobile phase, and analyzed by LC-MS/MS.

Results:

Bias & Precision:

Bias was <20% for Δ^9-THC and Δ^9-THCCOOH in liver homogenate and supernatant with a single exception of the low QC concentration for Δ^9-THC in liver homogenate (-29%). Within-run and between-run CV was <20% for Δ^9-THC and Δ^9-THCCOOH in liver homogenate and supernatant.

Ionization Suppression/Enhancement:

Δ^9-THC and Δ^9-THC-D_3 exhibited significant ion suppression (CV >20%) in both liver homogenate and supernatant, while Δ^9-THCCOOH and Δ^9-THCCOOH-D_3 showed both ion suppression and enhancement in these matrices. It was determined through method validation that both Δ^9-THC and Δ^9-THCCOOH would be reported qualitatively in solid tissue specimens.

Authentic Casework:

Estimated solid tissue concentrations (comprising of liver, skeletal muscle, and kidney) of Δ^9-THC and Δ^9-THCCOOH in homogenate and supernatant from 11 postmortem cases are presented. Noticeable quantitative differences were observed in both solid tissue homogenate and supernatant specimens despite evaluating from identical tissue samplings. These data from authentic postmortem casework also confirm earlier findings that both Δ^9-THC and Δ^9-THCCOOH show an

inconsistent tissue distribution, irrespective of whether homogenate or supernatant was analyzed.

Discussion: It is evident that the analytical technique used, the desired LOD/LOQ of the assay, and the physicochemical properties of the analyte(s) should be taken into consideration when making the determination to include solid tissue homogenate or supernatant within the scope of the method, especially when a decision is made to report those results quantitatively. Such analyses can result in potential recovery differences that may inadvertently under or overestimate drug concentrations. This recognition is crucial for accurate interpretation and comparison of analytical results, particularly in forensic contexts where the determination of tissue drug concentrations may hold significant implications. As the differences between drug concentrations in solid tissue homogenate and supernatant potentially extend to other drug classes, further studies are needed to evaluate other drug classes of interest. This study can also apply in cases where solid tissue is the only specimen submitted and where the toxicology laboratory is subsequently reporting either a qualitative or quantitative result.

P16

Clinical and Toxicological Presentation of an Accidental "Tranq Dope" Fatality

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Introduction: Xylazine is a veterinarian non-opioid tranquilizer that is not approved for human use. Clinical studies were conducted but terminated due to its severe adverse effects on the central nervous system and increased hypotension. It acts as an agonist at alpha-2 adrenergic receptors, which decreases the release of norepinephrine and dopamine in the central nervous system resulting in effects such as analgesia, sedation, and muscle relaxation. It does not respond to reversal effects of naloxone and is a known adulterant in heroin, cocaine, and illicitly manufactured fentanyl. The combination of fentanyl and xylazine is commonly referred to as "TranqDope". Here we report a case of a 41-year-old female with a history of illicit drug use found unresponsive and in cardiac arrest by emergency medical services after using "cocaine" an hour prior. She was transported to the hospital where substantial skin ulcerations were noted by emergency room personnel. The decedent was diagnosed with anoxic brain injury and never regained consciousness. Her hospital course was complicated by infectious endocarditis and kidney failure, and she died six days after admission.

Objective: To investigate the clinical and toxicological findings of a suspected cocaine toxicity exhibiting clinical presentations of xylazine use.

Method: Antemortem specimens from approximately one hour after hospital admission were collected by South Austin Medical Center in Austin, TX. Specimens were screened for volatiles utilizing headspace dual column gas-chromatography with flame ionization detection and drugs of abuse by immunoassay (ELISA). Qualitative drug screens were performed with a validated LC-QTOF-MS method for the identification of 350+ drugs following a protein precipitation. Xylazine was monitored since November 2020 and validated for the LC-QTOF-MS screen in June 2023. Presumptive positive analytes were confirmed and/or quantitated with validated LC-MS/MS methods following either solid phase extraction (cocaine and metabolites) or alkaline liquid-liquid-extraction (fentanyl, norfentanyl, xylazine) techniques. Postmortem specimens were not tested due to the prolonged interval between initial presentation and death.

Results: Autopsy findings demonstrated a well-developed, well-nourished adult female with multiple areas of ulceration of the anterior right forearm and both distal lower extremities measuring up to 20 cm. Routine toxicology testing identified ecgonine methyl ester (<20ng/mL) and benzoylecgonine (44ng/mL), along with fentanyl (42ng/mL), norfentanyl (130ng/mL), and xylazine (990ng/mL) in antemortem hospital blood. Following a thorough death investigation, autopsy, and toxicology, the cause of death was certified as "toxic effects of fentanyl, xylazine, and cocaine", with infectious endocarditis and hypertensive cardiovascular disease listed as contributing conditions. The manner of death was ruled an accident. Since May 2023, xylazine was detected in 12 additional cases: nine below the LOQ (5.0ng/mL), and three with a mean (range) concentration of 68±72ng/mL (14-150ng/mL).

Conclusion/Discussion: Despite the decedent's apparent use of what was thought to be cocaine prior to hospital admission, fentanyl and its metabolite and xylazine were detected and quantitated in antemortem blood in addition to cocaine metabolites. This case represents the highest xylazine concentration observed at the Travis County Medical Examiner to date. Skin ulcerations, such as those observed in this case, have been described in xylazine users and are known to develop in various areas of the body, irrespective of injection site. Recently, "TranqDope" has become popular amongst illicit drug users as the combination is known to prolong the effects of "feeling high." This case highlights the public safety threat of fentanyl and xylazine in the current illicit drug supply and the unintentional consequences of illicit drug formulations thought to be one substance but are in fact another entirely. However, without aggregate drug chemistry results, it is difficult to ascertain if their combination is a result of separate exposures or use of a combination product.

P17

Quantification of Daridorexant, Lemborexant, and Suvorexant in Whole Blood using Liquid Chromatography Tandem Mass Spectrometry (LC-MS/MS)

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Introduction: Suvorexant, lemborexant, and daridorexant are dual orexin receptor antagonists (DORAs) used to treat insomnia. Unlike other traditional sleep medications, these DORAs do not exhibit gamma-aminobutryic acid activity, which may contribute to lower abuse potential. Consequently, they are classified under schedule IV of the 1970 U.S. Controlled Substance Act. However, they continue to be drugs of forensic interest due to their accessibility, long half-lives, and potential risk for next-day residual drowsiness, impaired motor coordination, and decreased alertness. These CNS depressant effects may be particularly pertinent in driving impairment cases or drug-facilitated sexual assault investigations. Hence, it remains important to develop analytical methods capable of identifying these novel sedative hypnotics for both clinical and forensic toxicological testing. With that in mind, the objective of this study was to develop and validate an extraction and analytical assay that can quantify daridorexant, lemborexant, and suvorexant in whole blood.

Objectives: The goals of this research were: (1) to optimize an extraction that would yield high DORA recoveries with lower matrix effects in blood, and (2) to develop and validate an LC-MS/MS method to quantify the DORAs at low limits of quantification.

Methods: An acidic/neutral liquid-liquid extraction (LLE) using pH 3.6 sodium acetate buffer (0.4 M) and n-butyl chloride was optimized to isolate daridorexant, lemborexant, suvorexant, and the internal standard (ISTD) suvorexant-d_6 from bovine blood. An Agilent 1290 Infinity II Liquid Chromatograph coupled to an Agilent 6470 Triple Quadrupole Mass Spectrometer was used for quantitative analysis. Chromatographic separation was achieved using an Agilent Infinity Lab Poroshell 120 EC-C18 and its matching guard column at 35°C, with the gradient elution profile having a total run time of 8 minutes. Mobile phase A and B consisted of 0.1% formic acid in deionized water and in acetonitrile, respectively, at a flow rate of 0.4 mL/min. Electrospray ionization was operated in positive mode, and the mass analyzer configured to multiple reaction monitoring mode with one quantifier and qualifier transition for analyte and ISTD identification. The method was fully validated according to ANSI/ASB 036 guidelines. A proof-of-concept study was performed with authentic blood samples from adjudicated casework obtained from participating laboratories.

Results: All accuracy, precision and other validation parameters met acceptability requirements in ANSI/ASB 036. LLE recovery was >94% across all analytes. A quadratic 1/x model was utilized for daridorexant and lemborexant, while a linear 1/x model was used for suvorexant quantification. The calibration range was between 0.25-500 ng/mL for all analytes, with the LOD/LLOQ set at the lowest calibrator. Comparing between post-fortified extracts and neats (post extraction addition) at the low and high QC concentrations yielded matrix effects between -54.2% to -75.7%. Nonetheless, LODs were consistently reproducible and remain unaffected by ion suppression (n=10). Across all QC concentrations (0.75, 100, 450 ng/mL), bias ranged between -10.9% to 8.8%, while within-day and between-run %CV were <17.7% and <9.8% respectively. Two-fold dilution integrity studies (1:1 dilution of 500 ng/mL HQC to produce a target of 250 ng/mL) yielded a bias <-7.6%, with within-day %CV and between-run %CV both <7.6%. Exogenous/endogenous interferences were negligible for all positive controls fortified with 176 common drugs of abuse and negative controls. Re-injection of the blank following the highest calibrator was free from carryover. Extracts were stable beyond 48 hours when stored at 4°C. Suvorexant was identified in nine authentic blood samples, with concentrations ranging between 9-46 ng/mL.

Discussion: An analytical workflow was developed and validated for the quantification of suvorexant, and more novel DORAs daridorexant and lemborexant in blood. With increased prevalence of sedative hypnotics in impaired driving or drug-facilitated sexual assault, this method will support the identification of these emerging drugs in forensic investigations.

P18

Utility of a ketone body color test in identifying acetone-positive cases in vitreous humor compared to HS-GC/FID in postmortem toxicology

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Introduction: In the United States, diabetes mellitus was the eighth leading cause of death in 2021. One of the life-threatening complications of diabetes is diabetic ketoacidosis (DKA). DKA occurs when blood glucose is unable to be broken down for energy due to insufficient insulin. Instead, the liver uses lipolysis; fatty acids are released and broken down into acidic ketone bodies. Accumulation of ketone bodies can cause thirst, fruity-smelling breath, headache, nausea and vomiting, stomach pain, coma, and death. Postmortem analysis of ketone bodies, such as acetone, is necessary to determine if DKA contributed to death. Vitreous humor (VIT) is a desirable specimen for testing as it is less susceptible to postmortem changes and has good correlation to blood concentrations. Color tests for ketone bodies are available which give results in a matter of minutes. Ketone bodies such as acetone are also detected via headspace gas chromatography flame ionization detection (HS-GC/FID), a common analysis in postmortem toxicology for blood alcohol.

Objectives: Investigate utility in performing color tests in VIT to identify decedents with elevated ketone bodies. Do color tests catch cases otherwise missed by blood HS-GC/FID testing?

Methods: Medicolegal cases from 2018-2022 were queried and included if ketone body and alcohol testing were performed. Cases were excluded if either test was reported “specimen not satisfactory.”

One drop of VIT was applied to a K-Check tablet (Biorex Labs, Cleveland, OH) for ketones determination and results read after 2.5 min. Any purple color change was considered positive. Tablets detect 0.020-0.025 g/100mL of acetone in urine; an aqueous positive control at 0.080 g/100 mL was analyzed with case samples. Red- or brown-colored VIT were deemed not satisfactory for testing, as the sample color could interfere with visual assessment.

Acetone was quantitated alongside ethanol, methanol, and isopropanol on an Agilent 7697A headspace autosampler coupled with Agilent 8890 GC with dual FID. Separation was achieved isothermally (40°C) using DB-ALC-1 (30 m x 0.53 mm x 3 µm) and DB-ALC-2 (30 m x 0.53 mm x 2 µm) columns (Agilent Technologies, Santa Clara, CA). The linear range was 0.010-0.200 g/100 mL with limit of detection 0.010 g/100 mL. Per laboratory procedure, blood was tested initially, with subsequent VIT or urine testing when blood was positive for any volatile.

Results: Decedents were white (44.9%), 30.1% were black, 22.2% Hispanic, 2.7% Asian, and <1.0% unknown, aged <1 to 102 years old (60.7% were 35-64 years old), and mostly male (70.0%).

Of the 8767 cases that had K-Check and volatiles by HS-GC/FID testing, 438 were positive for acetone; 45.2% of these cases had a history of diabetes and 57.1% had diabetes listed in the cause of death. Of the positive cases, acetone detection relied on positive K-Check results for 20 cases (4.6%), meaning the initial blood test was negative for acetone and other volatiles.

VIT K-Check results were largely in agreement with acetone positive blood HS-GC/FID results. The prevalence of ketone body positive cases was 4.6%. Sensitivity, specificity, and efficiency were 89.6%, 98.1%, and 97.7% respectively. Negative predictive value was 99.5%; however, with a larger number of false positives, the positive predictive value (PPV) was 69.7%.

Discussion: In this study, the lower PPV value may be attributed to K-Check cross-reactivity with acetoacetate, which was not confirmed by HS-GC/FID, or to acetone distribution differences between blood and VIT. While K-Check provides quick and easy testing for ketone bodies, it is redundant if also running HS-GC/FID for volatiles. With less than 5.0% of cases relying on K-Check to identify ketone bodies, this testing may not add additional value to toxicologists or pathologists in death investigations

P19

Stability of Fentanyl in Fortified and Authentic Postmortem Blood

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Introduction: As one of the most frequently detected opioids in the United States in 2023, it is imperative that postmortem toxicology laboratories fully understand the implications of fentanyl's stability. Postmortem samples come to the laboratory in a variety of states of decomposition, yet there is a lack of information in the scientific literature detailing stability of drugs in purge or decomposing blood with the majority of stability studies being conducted in non-human blood products. By analyzing authentic postmortem samples or fortifying drug-free case samples with drug, a more accurate understanding of the stability of drugs such as fentanyl can be attained.

Objectives: The goal of this study was to evaluate the stability of fentanyl in (1) pooled positive fentanyl right chest purge and (2) fortified negative right chest purge post mortem samples stored under a variety of temperature conditions for six months.

Methods: Right chest purge fentanyl-positive (16.6 ng/mL) and fentanyl-negative samples were identified from the Jefferson County Coroner Medical Examiner's Office (JCCMEO) database within the prior 12 months and respectively pooled. All samples were originally collected in sodium fluoride, potassium oxalate grey top tubes, analyzed, stored in the -20° C freezer, and then later pulled, defrosted, and pooled for this study. Drug-free purge was fortified with fentanyl (25 ng/mL) and the two types of samples were stored as individual, 1 mL, aliquots at room temperature (20◦C), in the refrigerator (4◦C), or in the freezer (-20◦C) pending analysis. On the allotted day, triplicate aliquots from each storage condition were analyzed on an Agilent 8800 gas chromatograph (GC) system with a 5977 C mass selective detector (MSD) following solid phase extraction (SPE) with Clean Screen® DAU cartridges. Following the initial concentration evaluation, samples were analyzed on days 3, 7, 14, 30, 60, 90, 120, 150 and 180. Calibration curves were prepared from sheep's blood fortified with seven different concentrations of fentanyl (3-50 ng/mL) with fentanyl D5 as the internal standard.

Results: The six-month stability for both the purge positive and purge fortified samples showed that freezer storage outperformed both refrigerated and room temperature conditions, greatly increasing the stability of fentanyl in degraded samples. Using an 80% reference point from the original average concentration, room temperature and refrigerated purge positive samples were stable for 30 days, while the frozen counterpart was stable for 120 days. In the fortified purge samples, room temperature and refrigerated samples were stable for 14 days, while the frozen sample was stable for 60 days.

Discussion: The results of the stability study indicate that storage at freezing temperatures might reduce degradation risks and improve the dependability of fentanyl analysis in decomposed samples. Fortified samples may not reflect the true stability of a drug as it is removed from the natural environment and likely doesn't accurately reflect the nuances of protein binding and other enzymatic and cellular interactions. Legitimacy of the results in decomposed specimens has been a controversial topic with major implications for forensic investigations and establishing accurate cause and manner of death. Unfortunately, there is a paucity in the current literature of completed studies for stability of fentanyl in non-decomposed blood with which a comparison could be made. Preliminary data in the literature from an ongoing study shows stability of fentanyl at room and refrigerated temperatures up to 3 months and instability at freezing temperatures past 2 weeks. The current analytical techniques and specimen processing protocols will hopefully be affected by studies like this and improve the integrity of future analysis and lead to future studies of fentanyl. Altered storage policies and procedures for fentanyl analysis in the forensic context may result from additional investigation and validation of these findings.

P20

A Toxicological Investigation amongst the Homeless Population in Jefferson County, AL

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Introduction: Many homeless people battle with alcohol addiction, drug addiction, and/or mental illness, struggling to meet basic daily needs. Research into the cause of fatalities in homeless individuals is limited. Previous studies show that drug and alcohol related deaths are high in the homeless population. Currently, the patterns of drug use by the homeless population in Alabama remain understudied.

Objectives: In this study, we will document the prevalence of drug-related deaths in the homeless population of Jefferson County, Alabama, between 2012 and 2023, while distinguishing between overdose deaths and those attributable to chronic, addiction-related comorbidities and other causes of death.

Methods: The Jefferson County Coroner and Medical Examiner Office (JCCMEO) database was queried for postmortem data using the search terms “homeless,” “transient,” or “abandoned” which was then cross-checked for homeless decedents between 2012 and 2023. This collection of data was then analyzed for age, sex, race, type of drug, cause and manner of death, and number of days from last seen alive to found.

Results: There were 310 deaths among the selected population between January 2012 and May 2023. Ages ranged from 20-76 years, with 84% male and 15% female. There were 186 White, 6 Hispanic, 116 Black, 1 Asian, and 1 Other race in the cohort. Of the 310 cases, 43% were associated with drug toxicity of which the majority were polydrug. Fentanyl was detected in 51% of the cases, methamphetamine in 18%, heroin in 14%, cocaine in 10% and xylazine in 6%. The majority of deaths were accidental (57.5%) in nature with 21% being attributed to natural causes, 10% homicide, and 3% suicide. In the remaining 8.5% of cases, the cause of death was undetermined. In 23% of the natural deaths, chronic substance use/abuse was listed as a means of death. Fourteen individuals (4.5%) were found more than 5 days after they were last seen alive. On average, these decedents were found after 45 days (range 5-182 days). In comparison, of all assumed cases in 2023, 43 (3.5%) were found greater than five days from when they were last seen (range 5-107 days, average 18 days).

Discussion: In Jefferson County, AL, drug mortality rates are slightly higher for Black individuals, regardless of housing status. However, within the homeless population, mortality rates were highest in White males despite a higher Black homeless population. Over 50% of the cases had drugs associated with cause, means, and/or contributing factors to death. Further collection of data on drug use in the homeless population, including both postmortem data and statistics from overdoses in the emergency room or hospital admissions for unrelated reasons, could lead to improved insight into the patterns of drug use in the homeless population. With this information, first responders can be better informed to help those for whom it’s not too late and help the county and the state understand some of the problems faced by the homeless.

P21

Semi-quantitative multiplex screening of 29 drugs from a single blood sample on the Evidence MultiSTAT biochip analyser

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Introduction: Drug detection testing 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 using defined discrete test regions (DTRs). This 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 may be increased.

This study reports the application of this system to the simultaneous semi-quantitative detection of 29 drugs in blood samples. The application will be geared towards facilitating the screening process in testing laboratories who complete DUID and post mortem toxicology analysis. The assays of interest and cut-offs can be selected and validated, and two samples can be analysed in parallel.

Objectives: To validate a semi-quantitative multi-plex test utilising biochip array technology and suitable for use on the fully automated Evidence MultiSTAT biochip analyser. The test should be capable of detecting 29 drugs in blood samples.

Methods: Simultaneous competitive chemiluminescent immunoassays (n=29) using defined 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. The system software processes and reports the multiple semi-quantitative results generated.

The assays on the Randox ToxPlex Blood biochip array are Methamphetamine (range:2.15 - 295.68ng/mL, cut-off:20ng/mL), MDMA (range:5.39 - 690ng/mL, cut-off:40ng/mL), Amphetamine (range:7.10 - 444.52ng/mL, cut-off:20ng/mL), Tricyclic Antidepressants (range:6.39 - 1192.71ng/mL, cut-off:60ng/mL), 6MAM (range:0.26 - 33.28ng/mL, cut-off:5ng/mL), Opiates (range:2.04 - 903.73ng/mL, cut-off:10ng/mL), Oxycodone (range:0.26 - 82.45ng/mL, cut-off:10ng/mL), Oxazepam (range:0.63 - 80ng/mL, cut-off:10ng/mL), Clonazepam (range:0.63 - 80ng/mL, cut-off:10ng/mL), Barbiturates (range:3.75 - 480ng/mL, cut-off:50ng/mL), Cannabinoids (range:2.19 - 146.80ng/mL, cut-off:10ng/mL), Dextromethorphan (range:0.31 - 40ng/mL, cut-off:5ng/mL), Methadone (range: 0.38 - 57.41ng/mL, cut-off:10ng/mL), Cocaine/BZG (range:2.63 - 336.12ng/mL, cut-off:50ng/mL), Meprobamate (range:9.38 - 1200ng/mL, cut-off:150ng/mL), Tramadol (range:2.66 - 340.20ng/mL, cut-off:50ng/mL), Fentanyl (range:0.07 - 8.84ng/mL, cut-off:1ng/mL), Buprenorphine (range:0.13 - 16ng/mL, cut-off:1ng/mL), Propoxyphene (range:1.88 - 240ng/mL, cut-off:30ng/mL), Phencyclidine (range:0.72 - 92.24ng/mL, cut-off:10ng/mL), Zolpidem (range:0.63 - 80ng/mL, cut-off:10ng/mL), Ketamine (range:5 - 640ng/mL, cut-off:50ng/mL), Haloperidol (range:0.63 - 80ng/mL, cut-off:10ng/mL), Methaqualone (range:3.71 - 474.52ng/mL, cut-off:100ng/mL), Pregabalin (range:98.59 - 12620ng/mL, cut-off:1000ng/mL), Xylazine (range:0.54 - 69.12ng/mL, cut-off:8ng/mL), Ethyl Glucuronide (range:143.04 - 9790.68ng/mL, cut-off:600ng/mL), Acetaminophen (range:1.72 - 220µg/mL, cut-off:15µg/mL) and Salicylic Acid (range:6.25 - 800µg/mL, cut-off:50µg/mL).

Results: Limit of Blank (LOB) was determined by running 20 negative samples on 3 different analyzers (n = 60). The LOB was calculated by parametric (normal) or non-parametric (non-normal) statistics depending on distribution of samples. LOB achieved was at least 3 times lower than validated cut off for all assays on the array.

Between (inter) run precision and recovery was determined by assaying three spiked whole blood samples (at the validated cut off, at approximately -50% of validated cut off and at approximately +50% of validated cut off) across a minimum of 3 runs (n = 15 replicates). Assessment of recovery in spiked specimens is important for determining accuracy in sample matrix. Certified antigen standards were spiked into authentic negative whole blood matrix to complete the assessment. No extraction steps were completed. Recovery was calculated as follows:

Percentage Recovery = (mean analyte concentration observed in recovery sample / analyte concentration added) x100

Precision results were expressed as CV (%) and values were verified as <30% across all concentrations tested. Recovery achieved for all samples assessed was within 70 – 130%.

Discussion: Results show applicability of the simultaneous immunoassays applied to the Evidence MultiSTAT for the rapid semi-quantitative screening of a broad range of drugs from a single blood sample. User defined cut-off concentrations can be selected and validated to suit multiple drugs of abuse screening applications or the cut-off requirements per region.

P22

Development of an ELISA for the in vitro determination of Ethyl Glucuronide in oral fluid

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Introduction: Ethyl glucuronide (ETG) is a non-oxidative metabolite of ethanol. It is formed by the detoxifying pathway of alcohol elimination via conjugation with activated glucuronic acid (uridine-5'-diphospho-β-glucuronic acid). This is a minor pathway of alcohol metabolism and is reported to represent <1% of total alcohol elimination. However, ETG can be detected in body fluids for an extended period of time after the complete elimination of alcohol from the body. ETG has been shown to be a specific marker of ethanol intake in blood and urine.

Oral fluid testing has gained acceptance recently as an alternative matrix for detecting drugs in forensic and clinical settings. This is largely due to the less invasive nature of sample collection.

Objectives: To develop a one step, competitive ELISA for the screening of ETG in human oral fluid samples.

Methods: A competitive immunoassay was employed for the screening of ETG. The ETG capture antibody was immobilized and stabilized on a 96-well microtitre plate surface. The ELISA was standardised to ETG and, if present, in the sample, competed with horseradish peroxidase labelled ETG in the conjugate for a limited number of antibody sites on the microtitre plate. Enzyme substrate was then added to allow a colorimetric reaction to occur. Absorbances were read at 450nm and a standard curve was constructed based on these absorbances. Colour intensity is inversely proportional to the concentration of ETG present in the sample. The Randox ETG ELISA contains a pre-coated microtitre plate, conjugate, wash buffer, substrate and stop solution required to run the assay. It is validated for use with Quantisal and Intercept i2 oral fluid collection devices.

Results: By applying the provided sample dilution for each collection device, the ETG ELISA kit was developed to achieve a cut-off value of 20 ng/mL. Assay range for Quantisal collection device was 8.88 – 320 ng/mL and for Intercept i2 was 6.66 – 240 ng/mL.

The analytical evaluation of the ELISA showed the limits of detection as 8.88 ng/mL for Quantisal collection device and 6.66 ng/mL for Intercept i2 collection device (defined as the mean concentration of at least 20 negative samples plus 3 standard deviations).

Intra-assay precision was evaluated for 12 replicates of each standard level within a single run. Results were expressed as CV (%) and values were verified as <5% across all concentrations tested.

Recovery was determined by spiking authentic negative oral fluid pools collected using Quantisal and Intercept i2 collection devices. Each sample pool was spiked with certified ETG standard at -50% cut off concentration, at cut-off concentration and at +50% cut-off concentration. Three replicates of each sample was assayed across a total of 5 runs (n = 15 replicates) and recovery was calculated as follows:

Percentage Recovery = (mean analyte concentration observed in recovery sample / analyte concentration added) x 100

Recovery achieved for all samples assessed was within 74 - 104%.

Standardised against ETG, the cross-reactivity for ethyl-β-D-glucuronide was determined as 100%, for methyl-β-D-glucuronide was determined as 2.3% and for ethyl sulfate was determined as <0.01%.

Discussion: This one-step ELISA provides an excellent screening tool for the detection of ETG in human oral fluid samples. The Randox ETG ELISA (Catalogue number ETG10593) provides a valuable, convenient, and cost effective analytical tool for the rapid screening of oral fluid samples which have been collected using Quantisal or Intercept i2 collection devices. This is relevant because ETG is a specific marker of ethanol intake and oral fluid sample matrix is becoming more established in drugs of abuse screening due to the non-invasive nature of sample collection. The ELISA provides a preliminary analytical test result. A more specific alternative chemical method must be used to obtain a confirmed analytical result.

P23

Rapid and easy method for the determination of cocaine and six metabolites in postmortem hair

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Introduction: Cocaine is a widely abused drug globally, with an estimated 22 million users based on the 2023 UNODC report. Hair has been a biological specimen of increasing interest due to its long window of detection, coupled with a high stability, non-invasive sample collection, easy storage, and segmentation analysis, providing a thorough timeline of events. However, hair also has limitations including the possibility of external contamination. The analysis of metabolites could help to alleviate this issue, and to differentiate drug exposure from external contamination.

Objectives: The goal of this study was to develop and validate a quick, efficient, and sensitive method for the determination of cocaine and a wide panel of metabolites (benzoylecgonine (BE), cocaethylene (CE), norcocaine (NCOC), anhydroecgonine methyl ester (AEME), meta-hydroxy-cocaine (m-OH-COC) and meta-hydroxy-benzoylecgonine (M-OH-BE)) in hair samples by LC-MSMS.

Methods: Hair samples were decontaminated with a 3-step procedure, followed by homogenization via pulverization using a bead mill. A straightforward extraction followed, by using 10 mg of resultant hair powder, the respective internal standards (50 µL at 0.2 µg/mL of COC-d3, BE-d3,CE-d3, NCOC-d3 and m-OH-BE-d3) and 1 mL of methanol. The samples were incubated at 25 ℃ for 1 h in an ultrasound bath and then centrifuged at 25 ℃ and 5000 rpm for 5 min. The clear supernatant was transferred to a clean tube, and evaporated to dryness at 50 ℃ for 15 min. Analytes were reconstituted in 100 µL of mobile phase A:B, 98 : 2 (0.1% formic acid in 10 mM ammonium formate in water : 0.1% formic acid in acetonitrile). Reconstituted extracts were vortexed for 10 s and filtered by 0.2-mm PES nano filter vials. The filtered extracts were analyzed on a Shimadzu 8030 LC-MS/MS. A reverse phase Kinetex EVO C18 was used in gradient mode, with an oven temperature of 35 ℃, a flow of 0.2 mL/min and the total run time was of 5.5 min. The mass spectrometer operated in ESI positive mode, and data was acquired with MRM mode. The method was validated following the ANSI/ASB Standard 036 guidelines.

Results: Linearity was achieved with a linear model, weighing of $1/x^2$ and not forced through origin, across a 6-day period with 7 calibrators from 0.01 (LLOQ) up to 25.0 ng/mg. Precision was achieved with %CVs between 1.8 and 20.5% (n=15) while accuracy stayed between -20.5 and 16.7% (n=15). No endogenous or exogenous interferences (n=10) have been found. Matrix effects (n=13) were between -20.3 (%CV of 14%) and 27% (%CV of 8%) and extraction efficiency (n=6) and process efficiency (n=8) stayed between 94 – 119% and 85 – 185%, respectively. No carry over was observed and samples remained stable for 48 h in the autosampler. As a proof of concept, a total of 35 *postmortem* samples were analyzed (3 cm each), where 13 were deemed to be positive. From those, 7 were positive for both hydroxy metabolites. Cocaine concentrations ranged from 0.5 – 11.2 ng/mg, BE from 0.3 - 22.9 ng/mg, CE from 0.05 - 1.4 ng/mg, NCOC from 0.08 - 0.7 ng/mg, AEME from 0.05 – 1.5, m-OH-COC from 0.05 - 0.6 and m-OH-BE from 0.07 - 0.7 ng/mg.

Discussion: Extracts with the highest cocaine concentrations, also contained proportionately higher concentrations of the metabolites. All samples were collected at the Institute of Legal Medicine of São Paulo, Brazil and pertained to violent incident cases. Analytes in hair showed great stability provided these samples were also quantified six years ago, post-collection, and concentrations only varied in <3%. A rapid and easy method for the analysis of cocaine and its metabolites in *postmortem* hair has been developed, using only 10 mg of hair and a total run time of 5.5 min.

P25

LC-MS/MS Analysis of 14 Antipsychotic Drugs in Serum Utilizing Weak Cation Exchange SPE in a Miniaturized Microelution Format

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Introduction: Accurate quantitation is critical in Therapeutic Drug Monitoring (TDM) research to determine concentrations of drug analytes for proper dosing and reducing detrimental side effects. This communication describes an effective sample cleanup method for 14 of the most prescribed antipsychotics and their metabolites in serum: Aripiprazole, Chlorpromazine**,** Clozapine, Dehydroaripipprazole, Fluphenazine, Haloperidol, 9-OH Risperidone, Lurasidone, Promethazine, Quetiapine, Risperidone, Ziprasidone, Norclozapine and Olanzapine. The last two analytes in the panel are isobaric and therefore chromatographic differentiation is necessary. A parallel comparison of two 50 x 3.0 mm LC columns: Kinetex 2.6 mm Biphenyl core shell and modified fully porous Luna 3.0 mm Polar C18, demonstrated the superior retention and selectivity of the Kinetex Biphenyl column which also had excellent resolution of the isobaric pair. A mixed mode weak-cation exchange SPE using Strata-X-CW, in a Microelution 96-well plate format bypasses time-consuming evaporation and reconstitution of the extracted sample meeting the demands of a high throughput automated workflow.

Objectives: The objective of this work was to develop an efficient and reliable sample extraction method for the quantitative analysis of the fourteen antipsychotic drugs in serum samples.

Methods: An aliquot of 10 µL of serum pretreated with 200 µL of 25 mM ammonium format at pH 3.5 was loaded on a weak cation exchange SPE Strata-X-CW 96-well Microelution plate, that was conditioned and equilibrated with 200 µL each of Methanol and water respectively. Following two successive washes with 200 µL of ammonium formate buffer and 50% Methanol (aq, v/v), the sample was eluted with 100 µL of 5% Ammonium Hydroxide in Acetonitrile/Methanol (60:40, v/v). The eluted sample was diluted with 200 µL of 0.1% (aq, v/v) Formic acid (mobile phase A). Mobile phase B was 0.1% formic acid in methanol. Gradient elution using the Core-shell 2.6 µm, Kinetex Biphenyl 50x3.0 mm column provided a 5-minute chromatographic separation, that employed a step gradient successively from 20% to 40% to 80% and finally 95% of B in 3 minutes following a 1.5-minute equilibration. A SCIEX 6500 Triple Quad MS system was employed for MS/MS detection utilizing ESI under positive polarity.

Results: A variety of load, wash, and elution conditions were investigated for SPE method optimization. The representative analyte panel with reasonable hydrophobicity and basic characteristics, enabled application of an aggressive 50% Methanol wash for serum sample cleanup and resulted in absolute recovery 70% or greater from the Microelution SPE. Load and aqueous washes respectively were adjusted with the acidified 25 mM ammonium formate buffer. To maximize analyte recovery, a mix of Acetonitrile and Methanol (60:40, v/v) enriched with 5% ammonium hydroxide applied in elution, where dilution with mobile phase A allowed for direct injection of the extracted samples, with no evaporation. Additionally, it eliminated any chromatographic peak fronting and broadening issue particularly when larger volume injection made. Calibration curves over 100-fold dynamic range (5 ng/mL to 500 ng/mL) with 1/x weighting applied, resulted linear regression correlation value (R^2) ≥ 0.992 for all 14 analytes.

Discussion: We developed a sensitive, accurate and precise quantitation method for the 14-analyte antipsychotic panel, streamlining the workflow by employing direct injection of the extracted samples. The integration of Strata-X-CW Microelution SPE with the fast LC method using the Kinetex Biphenyl Column demonstrates an efficient workflow for the analysis of the antipsychotic panel.

P26

Validation of a Qualitative Immunoassay for 21 Drugs of Abuse in Oral Fluid on a Single Biochip Array

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Objectives: To validate a 21-analyte drugs of abuse screen in oral fluid utilizing the Randox Evidence+ analyzer with a custom biochip array.

Introduction/Background: Drug abuse is a major social and medical issue and usually requires costly interventions for the treatment and rehabilitation of abusers. This screening test is a rapid chemiluminescent assay for the qualitative detection of commonly abused drugs in human oral fluid samples. The oral fluid matrix collection allows for noninvasive and fully observable specimen collection and can be used for monitoring prescription drugs or controlled substances in various clinical and forensic settings.

Methods: A custom biochip array (Randox Laboratories Ltd.) included 21 discrete test regions (DTRs) for amphetamine, methamphetamine, opioids/opiates (targeting morphine, oxymorphone, oxycodone, hydrocodone, 6-monoacetyl morphine, tramadol, buprenorphine, fentanyl, and methadone), phencyclidine (PCP), cocaine (targeting benzoylecgonine), benzodiazepines (targeting oxazepam, lorazepam, and clonazepam), zolpidem, barbiturates (targeting phenobarbital), ethyl glucuronide, methylphenidate, and the marijuana metabolite, Delta-9-Carboxy-Tetrahydrocannabinol (THC-COOH). Blank synthetic oral fluid was spiked with different levels for accuracy samples, as well as in-house cutoff (Table 1), negative and positive assay controls. Patient samples and controls were collected using a Quantisal™ collection device from Immunalysis. The supernatant was analyzed on a fully automated Randox Evidence+® Analyzer. A robotic arm pipetted 60 µL of sample on to a biochip and then the analyzer performed a competitive chemiluminescent immunoassay. The results were generated as relative light units (RLUs).

Table 1. In-House Cutoff Values by Analyte

Group	Analyte	**Cutoff (ng/mL)**
Stimulants	Amphetamine	20
	Methamphetamine	10
	Cocaine Metabolite (Benzoylecgonine)	30
	Methylphenidate	100
Opioids	Morphine	10
	Hydrocodone	10
	Oxycodone	10
	6-acetylmorphine	5
	Oxymorphone	10
	Fentanyl	1
	Tramadol	5
	Methadone	10
	Buprenorphine	1
Marijuana Metabolite	THC-COOH	10
PCP	Phencyclidine	5
Benzodiazepines	Oxazepam	10
	Lorazepam	10
	Clonazepam	10
Non-Benzodiazepine	Zolpidem	10
Barbiturates	Phenobarbital	50
ETG	Ethyl glucuronide	300

Results/Discussion: Before validation, the following oral fluid collection technologies were tested for the best sample recovery and stability: Quantisal™, Salivette® (Sarstedt), and Intercept® (OraSure Technologies). The Quantisal™ collection device proved to be the most stable at each cutoff, positive, and negative level for all 21 analytes. The samples results were determined to be positive or negative based on the average RLU of cutoff controls for each analyte on an assay. The intra-assay and inter-assay precision had CVs (<13% and ≤21%, respectively) and 100% agreement for positive and negative controls along with no observed carryover for any of the 21 analytes. The sample recovery was excellent with minimal to no matrix suppression for all analytes. Accuracy was performed by comparing spiked analyte results from Randox Evidence+® Analyzer with the expected results and all the spiked samples were within 100% of interpretation agreement (positive or negative). When spiked accuracy sample results were compared with an external laboratory, some results were discordant due to known varying cross-reactivities and/or different cutoffs. In the end, these deviations did not affect clinical utility of the assay.

Conclusions: A novel drugs of abuse qualitative immunoassay in oral fluid using a custom biochip array was validated. The Quantisal™ collection device provides a convenient and easy method for sample collection. It is a convenient and efficient method of drug detection while using only a single sample preparation for each patient for screening of 21 drugs of abuse unlike other routine screening/ELISA methods which require separate assays for detection of these drugs. Therefore, this technology has potential to be utilized as high-throughput screening assay. Like all qualitative assays, the limitation of this test is that all positive results will need to be confirmed using a definitive methodology.

P27

Propoxyphene - Gone but not Forgotten

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Introduction: Propoxyphene is a central acting opioid analgesic that was patented in 1955 under the trade name Darvon for the treatment of mild to moderate pain. Products containing propoxyphene were discontinued in 2010 after a strong recommendation from the Federal Drug Administration due to concerns that the risks outweighed the benefits. Multiple studies demonstrated the potential for seizures as well as frequent heart rhythm abnormalities that were sometimes fatal. Randall Baselt's book, Disposition of Toxic Drugs & Chemicals in Man, references concentrations in propoxyphene-related deaths as low as 2 mg/L with several cases at concentrations lower than 1mg/L with no other drug present. Based on the ban of propoxyphene, the medication should not be identified in medical examiner cases, however storage and/or usage of old prescriptions provides an opportunity for self-medicating when experiencing chronic pain or executing a suicidal ideation. The continued presence of propoxyphene in Miami-Dade County is evident in cases screening positive for propoxyphene after 2010, and as recently as 2022.

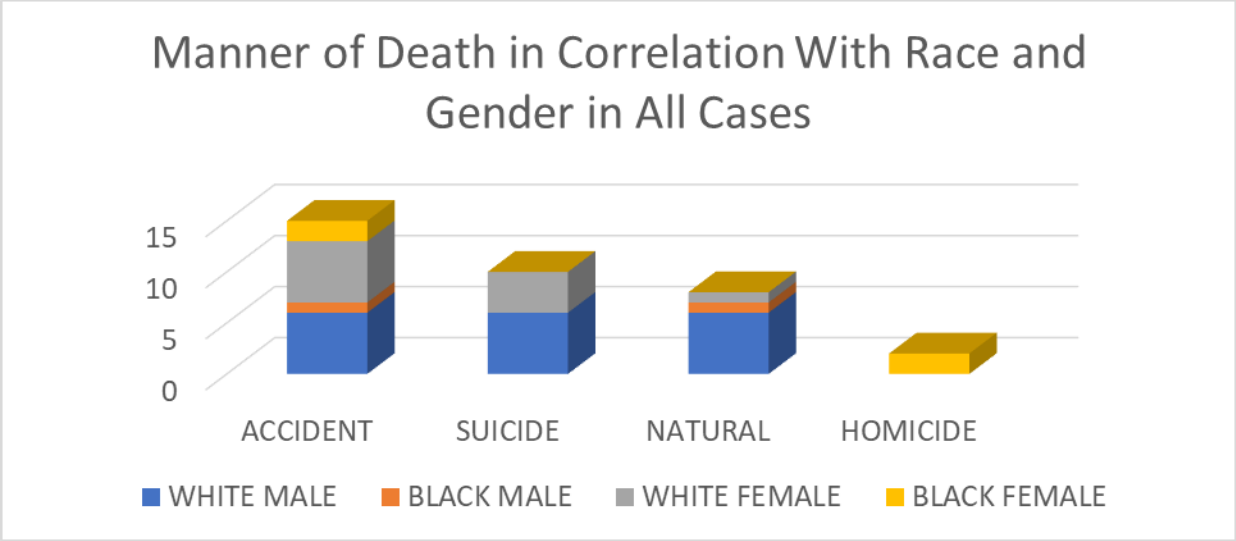
Objectives: The objective is to demonstrate the importance of including propoxyphene in the scope of laboratory testing by examining medical examiner cases in which propoxyphene was identified after the date of its ban in 2010. Additionally, a recent medical examiner case involving propoxyphene will be described.

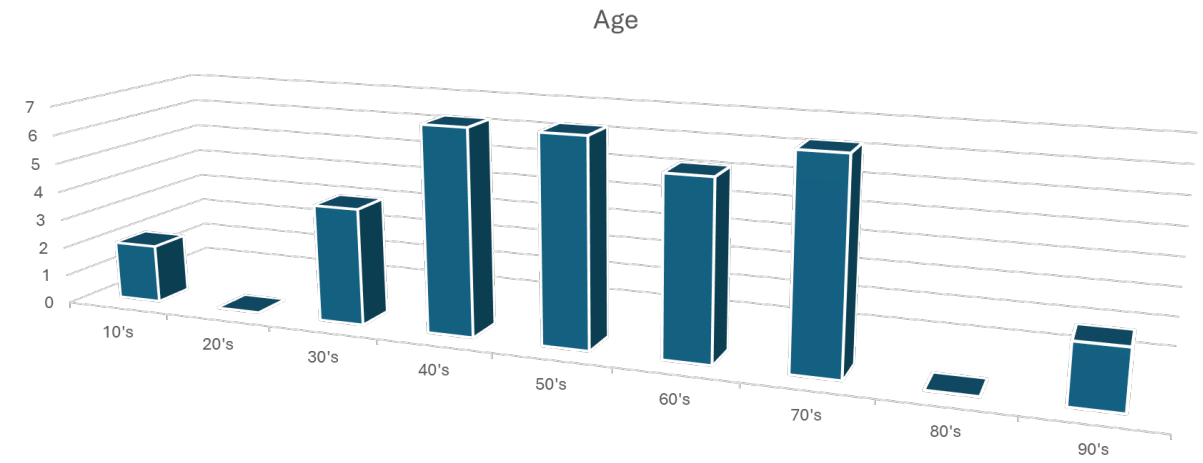
Methods: Propoxyphene and its major active metabolite, norpropoxyphene, are easily identified in basic drug screening methods. At the Miami-Dade Medical Examiner (MDME) Toxicology Laboratory, analysis of propoxyphene consists of solid phase extraction performed on blood, urine, liver, and gastric samples followed by detection utilizing a variety of instrumentation including gas-chromatography coupled with a nitrogen phosphate detector (GC-NPD), gas chromatography mass spectrometry (GC-MS), or liquid chromatography coupled to an ion trap mass spectrometer (LC-IonTrap-MSn). The laboratory's case management system was utilized to compile cases in which propoxyphene was identified during the period between 2010 and present. Cause of death (COD), manner of death (MOD), gender, age and race were included in the study.

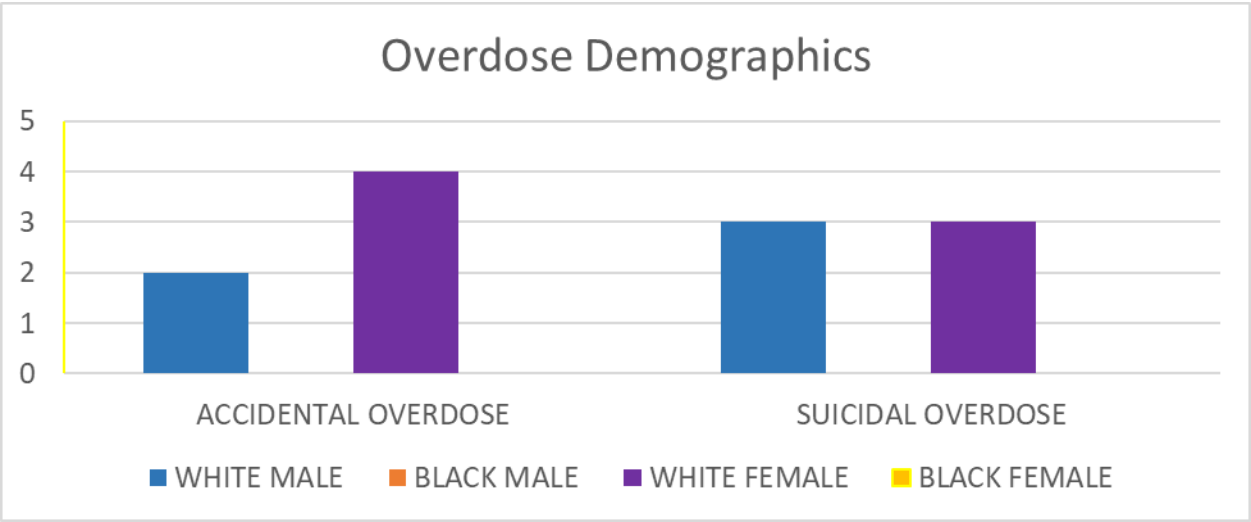
Results: Propoxyphene was detected in 35 medical examiner cases since 2010. Of these cases, 40% (14 cases) were accidental or suicidal overdoses with the majority (86%, 12 cases) listing propoxyphene in the COD. The decedents for these 12 cases were predominantly between the age of 40 to 60 years-old (67%), mostly female (60%), and white (100%) in race.

The most recent case at the MDME involves a 46-year-old white female with documented mental health issues found unresponsive in bed. Her autopsy showed severe atherosclerosis, hepatic congestion and fibrosis, chloesterolosis of the gallbladder, and discolored and hardened pancreas. The medical examiner initially certified the case as a natural death due to atherosclerotic coronary artery disease. However, toxicology testing revealed the presence of propoxyphene, clonazepam, butalbital, promethazine, zolpidem, and trazodone. The concentration of propoxyphene and norpropoxyphene in the iliac blood was 9.7 mg/L and 3.9 mg/L, respectively, which is well above the lethal range described in the literature. Bases on the toxicology findings, the medical examiner amended the cause of death to acute combined toxicity due to propoxyphene, and the manner of death as accident.

Full details can be seen in the charts below.







Discussion: The MDME Toxicology Laboratory continues to detect propoxyphene in case work despite its ban in 2010. Although surprising, several cases involving propoxyphene have been implicated in the cause of death recently which suggests that the drug is not completely obsolete but instead is lingering in people's medicine cabinets. Without the continual inclusion of propoxyphene in screening methods, these cases would potentially go unsolved.

P28

Analysis of Δ-8-THC, Δ-9-THC, and Their Metabolites in Whole Blood by LC-MS/MS

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Introduction: The testing of whole blood samples for tetrahydrocannabinol (Δ-9-THC) consumption is routine and has been around for many decades. Δ-9-THC is metabolized into 11-Hydroxy-Δ9-tetrahydrocannabinol (11-OH-Δ-9-THC) and further into 11-nor-9-carboxy-Δ-9-THC (Δ-9-THC-COOH). It is important to test for the parent and both metabolites to properly monitor for THC usage. As more isomers of Δ-9-THC become available on the market, testing becomes more complicated and novel methods are needed to achieve isomeric resolution. One such isomer, Δ-8-THC, is federally unregulated in the United States and readily available for purchase in many stores. This compound forms its own hydroxylated and carboxylated metabolites, (11-OH-Δ-8-THC and Δ-8-THC-COOH), that must be chromatographically resolved from their isomeric metabolites. The resolution of isomeric metabolites is key in reporting accurate specimen findings and poor resolution, especially when one isomer is in much greater abundance than the other, can result in quantitation issues and invalid data. In this study, an LC-MS/MS method was developed to adequately resolve all three isomeric pairs of compounds in whole blood.

Objectives: The primary objective of this study was to develop an LC-MS/MS method to adequately resolve isomeric pairs Δ-8-THC and Δ-9-THC, 11-OH-Δ-8-THC and 11-OH-Δ-9-THC, and Δ-8-THC-COOH and Δ-9-THC-COOH in a whole blood matrix.

Methods: An LC-MS/MS method was developed on a Raptor FluoroPhenyl 100 x 3.0 mm, 2.7 µm column using a mobile phase A (MPA) of water and a mobile phase B (MPB) of methanol, both acidified with 0.1% formic acid. The flow rate was 0.8 mL/min and the column temperature was 40°C. The gradient started at 64% B and held for 6.50 minutes, ramped up to 68% B at 6.60 minutes, and held at 68% B until 13.00 minutes. At 13.10 minutes the gradient ramped up to 100% B until 14.00 minutes, before returning to start conditions at 14.10 minutes and held until 16.00 minutes for ample re-equilibration. Whole blood samples were prepared using a liquid-liquid extraction. Extracts were dried down and reconstituted in 50:50 MPA:MPB. Δ-8/9-THC and 11-OH-Δ-8/9-THC were collected in ESI+ mode, while Δ-8/9-THC-COOH was collected in ESI- mode via polarity switching.

Results: All three isomer pairs were adequately resolved using the developed method. Calibration curves were tested ranging from 0.5-100 ng/mL for 11-OH-Δ-8/9-THC and Δ-8/9-THC, and 2.5-500 ng/mL for Δ-8/9-THC-COOH. Linearity was demonstrated using a $1/x^2$ weighted linear regression, and all analytes showed acceptable R^2 values. The method showed acceptable inter-day and intra-day precision and accuracy. No matrix or cross-analyte interferences with other structurally similar cannabinoids including CBD, exo-THC, and Δ-10-THC were observed.

Discussion: An LC-MS/MS method was successfully developed for reliable and accurate testing of Δ-8/9-THC isomers and isomer metabolites in whole blood. The method was determined to be rugged, sensitive enough to meet reporting guidelines for clinical and forensic toxicology laboratories, and of a reasonable analytical runtime. While C18 phase columns may show some selectivity for the three isomer pairs, full resolution of the isomers is needed for accurate quantitation. To achieve this resolution on a C18 phase would likely result in a lengthier analytical runtime. The FluoroPhenyl ligand shows selectivity for all three isomer pairs, allowing for adequate separation of the analytes in a reasonable analytical runtime.

P29

Multi-Class Drug of Abuse Rapid Screening in Urine, Plasma, and Oral Fluids Utilizing Paper Spray Mass Spectrometry

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Background/Introduction: Direct ionization mass spectrometry (DI-MS) has emerged as a valuable addition to toxicology workflows due to its high-throughput capability and rapid screening of analytes, surpassing traditional liquid chromatography (LC)-MS methods. Paper spray (PS)-MS is a specific type of DI-MS that involves spotting the sample onto a paper strip, followed by direct extraction and ionization at the ion source of the mass spectrometer. This is achieved by rehydrating the dried sample with an organic-aqueous mixture and applying a voltage with a spray solvent to facilitate ionization and introduction into the mass spectrometer.

PS-MS offers advantages over other DI-MS techniques for screening analytes in biological fluids. The presence of macromolecules and cell components in these fluids can be effectively "fixed" on the paper substrate due to the high organic content in the rewet and spray solvent, eliminating the need for sample cleanup required by other DI-MS methods.

In this study, we present a method for the rapid screening of over 50 drugs of abuse from various classes, including amphetamine, benzodiazepines, cocaine, sedatives, opioids, opiates, fentanyl analogues, and barbiturates. The screening is performed in urine, plasma, and oral fluids using a Thermo Scientific™ VeriSpray™ Paper Spray Ion Source coupled to a Thermo Scientific™ TSQ Altis™ Plus triple quadrupole mass spectrometer.

Objectives: Demonstrate rapid screening of multiple classes of drugs of abuse in urine, plasma, and oral fluids using a paper spray ionization source coupled to a triple quadrupole MS for forensic toxicology.

Methods: Drug-free human urine, plasma, and oral fluids were obtained from BioIVT. Non-labeled certified reference standards, as well as their corresponding isotope-labeled standards, were purchased from Cerilliant. The non-labeled standards (n=50) were combined to create a stock solution, which was then diluted with each matrix to generate 10-point calibration curves spanning from 0.5 ng/mL to 1000 ng/mL. A matrix mix consisting of 95 µL and 5 µL of internal standard (IS) was prepared and mixed with agitation. Subsequently, 10 µL of this solution was directly spotted onto a VeriSpray sample cartridge and allowed to dry overnight. All samples were spotted in triplicate, and the experiment was repeated on three separate days.

Data acquisition was performed using a one-minute method on a TSQ Altis Plus mass spectrometer operating in selected reaction monitoring (SRM) mode. One quantifier and one qualifier ion were monitored for each drug and its corresponding IS. Thermo Scientific™ TraceFinder™ 5.1 software was utilized for both data acquisition and data processing.

Results: Preliminary results demonstrated similar and satisfactory linearity for the majority of analytes in the presence of the three matrices. The limit of quantification (LOQ) (average around 10 ng/mL) was determined based on compounds with %diff within ± 20%, %CV below 15%, and R2 values above 0.98. The VeriSpray ion source - TSQ Altis Plus mass spectrometer exhibited LOQs that met or were below the ANSI/ASB Standard 121 requirement for urine screening in drug-facilitated crime investigations and the initial test cutoffs set by SAMHSA for oral fluids.

Conclusion/Discussion: Over 50 drugs of abuse from various classes in urine, plasma, and oral fluids were effectively analyzed with minimal sample preparation using the VeriSpray ion source coupled with a triple quadrupole mass spectrometer. The method demonstrated sufficient sensitivity specifically tailored for screening purposes in forensic toxicology and workplace drug testing.

P30

Old Dogs and New Tricks: A Fatal Case Involving Traditional Designer Drugs and an Emerging NPS Benzodiazepine

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Introduction: Methylenedioxymethamphetamine (MDMA) and methylenedioxyamphetamine (MDA - a minor metabolite of MDMA) are man-made psychedelic stimulants, often found in pill or powder formulations, that are typically ingested orally or by insufflation. Both are traditional "designer drugs" that were used recreationally for their sympathomimetic and hallucinogenic effects, especially at higher doses. However, with the emergence of new novel psychoactive substances (NPS) circumventing legislation, the occurrence of MDMA and/or MDA has decreased. Complicating matters further, what was once known as "ecstasy" or "molly" is not the "ecstasy" or "molly" of today. Often, it is a combination of other drugs including NPS, that may or may not contain MDMA or MDA. Here we report a case involving a 51-year-old male found unresponsive at home with obvious signs of death after sleeping all day following the ingestion of a liquid thought to be MDA with their partner. The decedent had a known history of chronic alcoholism and recreational use of Xanax, marijuana, MDA, cocaine, and hallucinogens.

Objective: To present the findings of a fatality involving an unusual route of administration of a traditional recreational drug that was suspected to be one drug, but in fact was another. This case report highlights the public safety threat of the current illicit drug market and the continued need to include traditional recreational stimulants, like MDA and MDMA, in toxicological testing despite the emergence of NPS.

Method: On the morning of the decedent's death, the decedent and their partner poured and drank what was reported to be liquid MDA into two shot glasses. Immediately following, the partner vomited, and the decedent repeatedly stated they did not feel well, eventually going to bed. The decedent was found 12 hours later, cold to touch, and unresponsive in bed. Scene findings revealed empty shot glasses, alcohol, unopened cigarettes, drug paraphernalia, and a small vial with an unknown substance located on the bedside table. Additionally, small baggies of all different colors containing white powdery substances were located in another room, along with fentanyl drug testing strips.

All postmortem specimens were collected at autopsy at the Travis County Medical Examiner in Austin, TX. Femoral postmortem blood was screened for volatiles utilizing headspace dual-column gas-chromatography with flame ionization detection and drugs of abuse by immunoassay (ELISA) for eight drug classes. Qualitative drug screening was performed with a validated LC-QTOF-MS method following an acetonitrile protein crash for the identification of 350+ drugs. Presumptive positive analytes were quantitated with validated liquid chromatography tandem mass spectrometry methods following various sample preparation techniques.

Results: Autopsy findings demonstrated a well-developed, overweight male with cerebral edema, a dilated and enlarged heart, and hepatic steatosis (fatty liver disease). Routine toxicology testing identified MDMA (3600 ng/mL), MDA (<40 ng/mL), bromazolam (160 ng/mL), benzoylecgonine (330 ng/mL), and ecgonine methyl ester (<20 ng/mL) in postmortem femoral blood. Through autopsy, investigative, and toxicological findings, the manner and cause of death was ruled an accidental death due to toxic effects of MDMA, bromazolam, and cocaine.

Conclusion/Discussion: The decedent believed they were taking a shot of MDA, however, toxicology identified MDMA and its metabolite (MDA), cocaine metabolites, and bromazolam (an NPS benzodiazepine) in the postmortem blood. This case highlights the public safety threat of the current illicit drug supply and the unintentional consequences of illicit drug formulations thought to be one substance but are another. Additionally, the resurgence of "old" recreational compounds coupled with emergence of "new" NPS create challenges for laboratories as they grapple with the demands of staying relevant and ensuring that law enforcement, death investigation, and public health stakeholders are properly served with updated and appropriate testing scopes.

P31

Aptamer-based on-site colorimetric tests for opioids

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Introduction: Opioids collectively cause over 80,000 deaths in the United States annually. The ability to rapidly identify these compounds in seized drug samples on-site will be essential for curtailing trafficking and distribution. Although chemical testing is quick and easy to perform, they are prone to false results, because the reagents employed in these tests react with generic functional groups that are shared by a broad range of compounds. Recently, portable Raman spectrometers have been employed to identify drugs like opioids. This approach is potentially very powerful because each molecule has its own unique Raman spectrum, making it highly specific. However, they face difficulties with highly impure, heavily adulterated drug samples, and generally have poor detection limits, precluding the detection of drugs such as fentanyl, which may be present at only trace amounts.

Objectives: Nucleic-acid-based bioreceptor known as aptamers have the potential to overcome current challenges associated with drug detection. They are oligonucleotide receptors that bind to specific molecules with high affinity. We utilized an in vitro method termed systematic evolution of ligands by exponential enrichment (SELEX) to isolate DNA aptamers that bind to either fentanyl or heroin and then used these isolated aptamers to develop colorimetric sensors for these targets.

Methods: We first used the SELEX technique along with counter selection strategy to isolate aptamers that bind to fentanyl or heroin with excellent specificity. High-throughput sequencing (HTS) of the selection pools revealed a variety of aptamer candidates, which we subsequently screened for their binding properties using an exonuclease digestion fluorescence assay.

Results: We determined that the isolated DNA aptamers bind to fentanyl with high affinity and specificity. The heroin aptamer specifically binds the target and has no response to the interferents found in seized substances such as cutting agents, other illicit drugs, endogenous compounds in the opium plant, and opioid receptor antagonists. We then characterized the binding affinity of these aptamers using the gold-standard approach isothermal titration calorimetry and utilized a set of high-quality aptamers to develop dye-displacement assays. We observed that our aptamer-based dye-displacement assay could detect fentanyl and heroin by the naked eye via a color change that occurs within seconds, with no response to structurally similar drug molecule dextromethorphan, cocaine, methamphetamine, acetaminophen, and methadone. To demonstrate the utility of our assay, we compared its sensing performance to the Marquis Test. Our aptamer assay could identify minute quantities of fentanyl (8%) in heroin samples directly. In contrast, the Marquis test completely failed these tests. Finally, we simplified assay procedures to an extent such that laypersons such as law enforcement officials can easily perform opioid testing outside of laboratory settings.

Discussion: Our aptamers and assays developed here will be of great use for the screening of opioids in seized substances for forensic drug analysis. The assay is therefore significant because it has solved a longstanding problem that no other in-field analytical approaches could address. We also demonstrated that our test can be performed by simply mixing the aptamer-dye solution with a crushed and dissolved drug sample and observing an immediate color change by naked eye. We believe that this assay is therefore feasible for first responders and law enforcement personnel to augment drug interdiction efforts and prevent harm associated with the consumption of opioid-adulterated substances.

P32

Quantitative Analysis of Δ8- and Δ9-Tetrahydrocannabinol Metabolites and Isomers: A Rapid Assay in Urine by LC-MS/MS

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Introduction: In the United States, various states and jurisdictions have legalized the use of cannabis for medical and/or recreational purposes. An increasing number of cannabis-related products have become available and entered the market, particularly those containing cannabidiol (CBD) and Δ8-tetrahydrocannabinol (Δ8-THC). Analytical screening and confirmatory methods for cannabinoids in urine samples have been described extensively in the literature. However, methods providing adequate resolution for distinguishing interferences from THC positional isomers are needed.

Objectives: The aim of this project was to develop and validate a liquid chromatography with tandem mass spectrometry (LC-MS/MS) method to quantitate a broad panel of cannabinoids in authentic urine specimens.

Methods: The method was optimized to quantitate Δ8- and Δ9-tetrahydrocannabinol (Δ8-THC and Δ9-THC), 11-hydroxy-Δ8-THC and 11-hydroxy-Δ9-THC (11-OH-Δ8-THC and 11-OH-Δ9-THC), 11-nor-Δ8-carboxy-THC and 11-nor-Δ9-carboxy-THC (Δ8-THC-COOH and Δ9-THC-COOH), CBD, 7-carboxy-cannabidiol (7-COOH-CBD), cannabigerol (CBG), and cannabinol (CBN) in urine. A dual enzymatic and chemical hydrolysis was optimized to cleave both the ether and ester glucuronides from the compounds. Internal standards were added to the samples (500 uL) along with 100 uL of Finden Kura Biotech BGTurbo® Enzyme, and incubated at 50°C for 30 min. A second hydrolysis at 50°C for 10 min was performed after the addition of 50 uL of 10.0 N sodium hydroxide. Finally, the samples were diluted with 500 uL of 0.1% formic acid in acetonitrile before being filtered using UCT Clean Screen FASt Extraction Columns (200 mg/3 mL) and transferred into vials for analysis. The samples were analyzed using a Thermo Scientific Vanquish™ Flex UHPLC System coupled with a Thermo Scientific TSQ Altis™ triple quadrupole mass spectrometer. The method was validated according to the American Academy of Forensic Sciences Standards Board (ASB) Standard 036. Validation parameters included selectivity; stable isotope internal standard and common analyte interferences; calibration model; carryover; ion suppression/enhancement; lower limit of detection (LLOD) and lower limit of quantitation (LLOQ); accuracy (bias) and precision; dilution integrity; and processed extract stability. The validated assay was then used to evaluate urine samples collected over various time points from female patients (n=69) enrolled in a study on assessing prevalence of marijuana/CBD use during pregnancy from November 2022 to May 2024.

Results: The Δ^8- and Δ^9- isomers were chromatographically resolved and successfully separated. For all analytes, the LLOQ was determined to be 10 ng/mL, and the upper limit of quantitation (ULOQ) was 1000 ng/mL. No interferences from matrix, internal standard, or common drug analytes were observed. Bias and precision were within the acceptable ± 20% criteria range. Matrix effects, extraction recovery, and process efficiency were deemed acceptable for all analytes. Dilution integrity was evaluated using highly concentrated samples injected after preparation and after 10x dilution, and analyte concentrations were within ± 20% of the target concentration. The most frequently detected analyte was Δ^9-THC-COOH in the authentic samples (55.1%), with a median concentration of 278.9 ng/mL. Other analytes, Δ^9-THC and 11-OH-Δ^9-THC, were detected with a median concentration of 42.4 ng/mL (n=5) and 65.7 ng/mL (n=34), respectively. Δ^8-THC-COOH was detected in n=3 specimens (4.3%), with a median concentration of 25.5 ng/mL.

Table 1. Data set for the studied compounds.

Analyte	Range (Median) ng/mL
Δ^8-THC-COOH (n=3)	15.6-679 (25.5)
Δ^9-THC (n=5)	12.7-76.2 (42.4)
11-OH-Δ^9-THC (n=34)	12.4-2582 (65.7)
Δ^9-THC-COOH (n=38)	12.6-5663 (278.9)
CBD (n=1)	54.2
7-COOH-CBD (n=2)	22.8-37.2 (30.0)
CBG (n=10)	12.4-233 (49.4)

Discussion: Given the psychoactive potential and availability of cannabis-related products, distinguishing and monitoring Δ^{8}- and Δ^{9}-THC isomers in toxicology laboratory routine workflows has become necessary. The present study aimed to provide the toxicological community with a rapid LC-MS/MS method for the analysis of a broad panel of cannabinoids in urine.

P33

Analysis of Phosphatidylethanol (PEth) in postmortem decomposition blood samples with corresponding vitreous humor ethanol concentrations

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Introduction: Phosphatidylethanols (PEth) constitute specific non-oxidative category of phospholipid ethanol metabolites, arising from the interaction between ethanol and phosphatidylcholine facilitated by phospholipase D. Among PEth metabolites, those incorporating palmitic and oleic or linoleic acid (16:0/18:1 and 16:0/18:2) have been identified as the most prevalent. PEth is becoming a promising marker for long-term alcohol consumption, remaining detectable in the body for up to 20 days following abstinence. It has also found utility as a marker for alcohol abuse disorder.

PEth formation may be influenced by previous ethanol exposure, and the amounts and activity of phospholipase D isoenzymes, which vary among organs. Detection of PEth in postmortem tissue/fluids may provide reliable evidence of previous alcohol exposure in investigation of alcohol-related deaths and the possibility of post-mortem production of ethanol and/or PEth.

Vitreous humor, being encapsulated in the eyeball, is less subject to post-mortem ethanol generation and ethanol levels tend to remain constant.

Presented is an examination of PEth concentrations in postmortem decomposition blood (PDB) samples, and corresponding ethanol concentrations in the decomposition blood and vitreous humor samples.
Objectives: The objective of this study is to ascertain the suitability of PEth metabolites (16:0/18:1 and 16:0/18:2) for identifying alcohol use/abuse in PDB samples.

Method: PEth metabolites (16:0/18:1 and 16:0/18:2) were quantified in PDB samples using a modified previously published method. Briefly, 100µL of calibrators (ranging from 5 to 5000ng/mL), controls (0, 15, 200, and 750ng/mL), or sample were aliquoted into microcentrifuge tubes along with 25µL of PEth 16:0/18:1-d5 (internal standard, 250ng/mL in H_2O: isopropanol (1:1)), and 100 µL of H2O: acetonitrile (80:20), then thoroughly mixed. The samples were loaded onto a Biotage ISOLUTE® SLE+400µL Sample Plate and eluted with three aliquots of 700µL ethyl acetate: isopropanol (95:5). The eluates were evaporated to dryness and reconstituted with 100µL of isopropanol. Analysis was performed using a Waters TQS-micro ultra-performance liquid chromatography-tandem mass spectrometry system, equipped with a Luna Phenyl-Hexyl column (50mm×3mm×5µm). The mobile phase consisted of A: 2mM ammonium acetate, B: methanol/acetone (95/5). Method validation was conducted according to ANSI/ASB Standard 036, covering evaluations for linearity, precision, accuracy, selectivity, post-preparative stability, interference, carryover, and limit of detection.

De-identified PDB samples from 34 cases were submitted for PEth analysis (subclavian (n=8); heart (n= 5); central (n= 2); femoral (n= 12) and “decomp” (n= 7). Ethanol concentrations in both blood and vitreous samples were determined using a validated headspace gas chromatography method.

Results: PDB samples collected from a known site (n= 27) and vitreous humor (n= 25) were positive for ethanol (0.012-0.338g/dL) and (0.010-0.394g/dL), respectively. PEth 16:0/18:1 (n= 25), and PEth 16:0/18:2 (n= 23) were detected in 23 PDB samples, which had corresponding ethanol-positive vitreous samples. All PDB samples, with no indicated site of collection, were negative for both PEth 16:0/18:1 and PEth 16:0/18:2. In the remaining 9 PDB samples, the vitreous humor was ethanol negative, the blood samples were ethanol positive (0.011 – 0.149g/dL) and 2 blood samples were positive for PEth 16:0/18:1 and PEth 16:0/18:2. Postmortem production of ethanol was suspected in 9 cases.

Discussion: The findings from this study, albeit based on a limited sample size, suggest that PEth can be detected in postmortem decomposition samples. PEth does not seem to form when postmortem ethanol production is suspected. Nine blood ethanol positive samples showed evidence of possible post-mortem ethanol production (vitreous ethanol negative) and only 2 had detectable PEth. PEth by itself, may not be a reliable biomarker for identifying ethanol abuse disorder in postmortem analysis.

P35

An overview of mitragynine and fentanyl positive postmortem casework in Dallas County

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Introduction: In August 2022, Dallas County Southwestern Institute of Forensic Sciences (SWIFS) revalidated its existing Cocaine, Opiates, and Metabolites (CocOps) quantitation method by liquid chromatography tandem mass spectrometry (LC-MS/MS) to include the quantitation of mitragynine and other analytes. Prior to this, mitragynine was reported qualitatively from a comprehensive drug screen by liquid chromatography time of flight mass spectrometry (LC-QTOF-MS). The revalidated method allowed for simultaneous quantitation of 24 analytes, including mitragynine and fentanyl. SWIFS has reported 28 postmortem cases that were positive for both fentanyl and mitragynine from the updated method.

Objectives: This presentation will provide information regarding the prevalence of mitragynine and fentanyl in postmortem casework in Dallas County from August 2022 through March 2024.

Methods: Fentanyl and mitragynine are both routinely identified at SWIFS in a comprehensive LC-QTOF-MS/MS screen and quantitated using LC-MS/MS. Crystal Reports software was used to generate a dataset for reported CocOps results from August 2022 through March 2024, which was further narrowed to include only blood positive results containing fentanyl and mitragynine. The demographics of the deceased individuals (race, gender, and age), scene observations, and cause and manner of death are reported in death investigation and medical examiner reports.

Results: During this time period, approximately 2900 postmortem cases were quantitated using this updated method. Of these cases, there have been 28 postmortem cases involving a combination of mitragynine and fentanyl reported by SWIFS. From the data collected, the demographics of the deceased can be summarized as primarily white males in their mid-30s. Of these cases, 26 of the deceased individuals had a history of drug use, drug paraphernalia was found at the scene, and/or it was suspected that an overdose occurred. The cause of death for every case was ruled as accidental by the medical examiners, with the exceptions of one case ruled as suicide and one case ruled as homicide. The manner of death for 23 of the cases specifically cited toxic effects of fentanyl and mitragynine. Fentanyl toxicity on the death certificate is a requirement of Texas House Bill 6 for the purposes of the death certificate and criminal penalties for certain controlled substance offenses and increase the criminal penalty.

	Fentanyl Concentration (ng/mL)		**Mitragynine Concentration (ng/mL)**	
Year	**Average**	**Median**	**Average**	**Median**
2022 (n=7)	7.6	6.0	117.3	79.3
2023 (n=17)	15.5	10.1	99.7	85.0
2024 (n=4)	9.1	5.0	1138.8	1038.7
Overall	12.6	8.0	270.9	116.4

Discussion: Due to the opioid-like effects at higher concentrations and its accessibility due to its legal status, the prevalence of mitragynine is unsurprising in the post-opioid epidemic world. As the fentanyl epidemic continues and the rise in concentration and prevalence increases, SWIFS expects to continue reporting fentanyl and mitragynine combination cases in its postmortem toxicology casework.

P36

American Molecular 10 mL Tubes, A Candidate for Human Performance Testing

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Introduction: Traditionally, grey top Becton, Dickinson (BD) and Company Vacutainer tubes are preferred for forensic toxicology testing. The Kansas Bureau of Investigation (KBI) Toxicology Laboratory was provided with 10 mL grey top blood tubes manufactured by American Molecular. The KBI Toxicology Laboratory participated in the testing of these tubes, focusing on blood ethanol stability over a six month period of time. Testing was completed on a total of 40 tubes while stored at room temperature and in refrigeration.

Objectives: Test American Molecular blood tubes for the stability and validity of ethanol in blood for forensic toxicology purposes.

Methods: Blood was drawn from KBI laboratory personnel directly into the blood tubes using aseptic phlebotomy techniques to ensure that there was no mixture of preservatives or anticoagulants from other brands of tubes. Each tube was sorted after the blood collection into three categories: low (3-5mL), medium (4.5-7.5mL), or high (9.5-10.5mL).

Within two days of spiking the blood tubes with ethanol, each tube was analyzed using the KBI's validated blood alcohol method. This was done to establish a baseline of known concentrations. One sample per volume category was intentionally left blank to exhibit no ethanol formation in tubes. Samples 1-26, all high and low volume samples, were spiked with ethanol and tested monthly to determine if there were any changes in concentration over time. These samples were refrigerated when not being tested. Samples 27-33, first half of the mid-range volume samples, were tested for their initial concentration and refrigerated undisturbed for 6 months before being tested again. Samples 34-40, second half of the mid-range volume samples, were tested for their initial concentration and left undisturbed at room temperature for 6 months before being tested again.

Results: Each tube was tested in duplicate and averaged. The exceptions included samples 01 and 03, as they did not contain enough volume to test on month 6. The final concentrations from month six were compared to the initial concentrations to determine if there was any change over time. The results in Table 1 and Table 3 with an asterisk indicate that one of the two vials did not pressurize, leaving one result for the month for that sample.

Table 1 shows the tubes that were tested every 6 months and refrigerated when not being tested. Concentrations are reported as g/100mL of blood.

Table 1: 6 Months of Testing Results

Sample Name	Starting Volume (mL)	Initial	Month 1	Month 2	Month 3	Month 4	Month 5	Month 6	Conc. Change	% Change
01	3.0	0.000	0.000	0.000	0.000	0.000	0.000	-	0.000	0
02	4.0	0.030	0.025	0.026	0.026	0.025	0.022	0.021	-0.009	-30
03	2.5	0.028	0.023	0.023	0.025	0.023	0.020	-	-0.008	-29
04	3.5	0.065	0.061	0.061	0.060	0.060	0.056	0.053	-0.012	-18
05	3.5	0.067	0.062	0.062	0.062	0.061	0.057	0.054	-0.013	-19
06	4.0	0.071	0.068	0.067	0.066	0.069	0.063	0.060	-0.011	-15
07	4.5	0.066	0.064	0.065	0.063	0.064	0.060	0.058	-0.008	-12
08	3.5	0.074	0.073	0.071	0.072	0.074	0.069	0.065	-0.009	-12
09	4.0	0.076	0.074	0.076	0.074	0.074	0.070	0.069	-0.007	-9
10	5.0	0.147	0.146	0.148	0.145	0.145	0.140	0.134	-0.013	-9
11	4.5	0.132	0.126	0.131	0.127	0.133	0.124	0.121	-0.011	-8
12	4.0	0.270	0.269	0.271	0.262	0.269	0.258	0.248	-0.022	-8
13	5.0	0.289	0.290	0.293	0.280	0.296	0.278	0.279	-0.010	-3
14	9.5	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0
15	10.0	0.030	0.025	0.026	0.026	0.025	0.023	0.020	-0.010	-33
16	10.0	0.029	0.026	0.026	0.026	0.026	0.023	0.022	-0.007	-24
17	10.5	0.062	0.061	0.062	0.060	0.059	0.056	0.056*	-0.006	-10
18	10.5	0.064	0.061	0.061	0.060	0.063	0.058	0.057	-0.007	-11
19	10.5	0.077	0.074	0.076	0.073	0.074	0.072	0.070	-0.007	-9
20	10.5	0.078	0.074	0.074	0.076	0.074	0.071	0.069	-0.009	-12
21	10.5	0.085	0.083	0.085	0.082	0.082	0.078	0.079	-0.006	-7
22	10.5	0.083	0.081	0.080	0.079	0.081	0.078	0.076	-0.007	-8
23	10.5	0.134	0.132	0.131*	0.130	0.133	0.128	0.127	-0.007	-5
24	10.5	0.141	0.141	0.145	0.138	0.143	0.136	0.136	-0.005	-4
25	10.5	0.273	0.277	0.283	0.267	0.271	0.273	0.268	-0.005	-2
26	10.5	0.281	0.284	0.287	0.288	0.281	0.277	0.279	-0.002	-1

Table 2 shows the tubes that were left in the refrigerator and tested 6 months after the original test date. The concentrations in Table 2 are reported as g/100mL of blood.

Table 2: Refrigerated samples					
Sample Name	Starting Volume (mL)	Initial	Month 6	Conc. Change	% Change
27	5.0	0.000	0.000	0.000	0
28	4.5	0.029	0.024	-0.005	-17
29	6.5	0.062	0.058	-0.004	-6
30	7.5	0.077	0.068	-0.009	-12
31	5.0	0.077	0.071	-0.006	-8
32	5.0	0.140	0.136	-0.004	-3
33	6.5	0.283	0.275	-0.008	-3

Table 3 shows the tubes that were left in the laboratory at room temperature and tested 6 months after the original test date. The concentrations in Table 3 are reported as g/100mL of blood.

Table 3: Room temperature samples					
Sample Name	Starting Volume (mL)	Initial	Month 6	Conc. Change	% Change
34	7.0	0.000	0.000	0.000	0
35	4.5	0.029	0.006	-0.023	-79
36	6.0	0.059	0.047*	-0.012	-20
37	7.5	0.074	0.057	-0.017	-23
38	4.5	0.075	0.056	-0.019	-25
39	5.5	0.143	0.126	-0.017	-12
40	6.0	0.263	0.250	-0.013	-5

The concentration change for low volume samples (samples 1-13) is -0.011 ± 0.004 g/100mL and high volume samples (samples 14-26) is -0.006 ± 0.002 g/100mL. The concentration change for refrigerated samples (mid-range sample 27-33) is -0.006 ± 0.002 g/100mL and room temperature samples (mid-range samples 34-40) is -0.016 ± 0.003 g/100mL excluding the outlier.

Discussion: Based on the above results, the KBI Laboratory's Toxicology Section finds the American Molecular tubes are a good candidate for use in human performance testing for ethanol analysis. There was one outlier in a low volume sample that was kept at room temperature (sample 35). The outlier was deemed not of concern to our laboratory because the starting concentration was below the Kansas per se limit of a 0.08 g/100mL of blood, and samples are generally not stored outside of refrigeration for a 6 month period in forensics casework. The greatest percent change was for the lower concentration samples.

P37

Foam Cones and Opioids – Part Deux

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Introduction: Foam cones have been associated with opioid-related deaths, but the presence is not specific to opioid-related deaths. At the 2023 SOFT Conference, data were presented supporting a strong correlation between the presence of foam/foam cones and opioid-related deaths, as 72.8% of 966 cases with foam reviewed from 2012 – 2023 had an opioid-related cause of death. However, during the same time span, only 16.0% of the 4,374 opioid-related had a foam/foam cone noted at the scene. This observation prompted further investigation into whether specific opioids are more or less likely to be associated with foam at the scene and also how foam at the scene relates to heavier lungs.

Objectives: Evaluate opioid-related cause of death cases from 2012 – 2023 for drug-specific trends for the presence of foam at the scene. Additionally, evaluate any trends between combined lung weights and presence of foam at the scene.

Methods: Cases from 2012 - 2023 were queried for scene investigator notes of foam or foam cone and an opioid-related cause of death. These cases were then pruned to remove cases that did not receive a full autopsy, were noted as decomposed, had a hospital delay, and a decedent's age <18 years old. After filtering, 2,907 opioid-related death cases were evaluated. The opioid drugs were grouped into three categories: traditional opioids, fentanyl and related analogues, and other opioids. The traditional opioids included codeine, heroin, hydrocodone, hydromorphone, morphine, oxycodone, and oxymorphone. Other opioids included methadone, mitragynine, tramadol, and U-47700. The combined lung weights were measured in grams.

Results: An initial review of 246 random cases from the total 2,907 revealed the following observations. Only 12.6% attributed the cause of death solely to an opioid; all others had another substance listed from another drug class. From the 246 cases, 130 cases listed a traditional opioid, 131 cases listed a fentanyl or related analogue, and 29 listed another opioid in the cause of death. For traditional opioids and other opioids, 60.0% and 58.6% of the cases had foam noted at the scene, respectively, compared to only 48.0% of the fentanyl and related analogue compounds.

To evaluate combined lung weights with the presence of foam at the scene, a random sub-set of 15 cases were selected and reviewed only based on whether foam was or was not present at the scene. Average combined lung weights were slightly higher in cases where foam was noted at the scene, 1379.5 g (n = 10, standard deviation – 369.6 g), compared to when it was not noted at the scene, 1160.0 g (n = 5, standard deviation – 398.0 g). However, there is no statistical difference between the averages. From this sub-set, other autopsy findings included that if foam was not noted at the scene, there was also no foam noted in the airway or lungs. But if foam was noted at the scene, 70% of the sub-set of cases also had autopsy findings noting that foam was also present in the airway or lungs.

Discussion: Initial case review indicates that there is no statistically significant association between specific opioids and foam at the scene. However, foam noted at the scene is slightly less prevalent with fentanyl and fentanyl related as compared to other opioids. Additionally, the foam noted at the scene appears to correlate with heavier combined lung weights, but, in a limited number of cases, there was no statistical difference between the average combined lung weights in cases where foam was or was not identified at the scene. These findings may be helpful in understanding scene and autopsy findings in the context of the changing novel drug landscape.

P38

Unraveling Drug Facilitated Sexual Assaults: Insights from Harris County, Texas, 2023

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Introduction: Drug facilitated sexual assaults (DFSA) pose significant challenges to toxicology analysis due to the diversity of drugs involved and low expected concentrations. Factors such as delayed reporting and continued metabolism further complicate investigations. Despite traditionally being seen as crimes against females, societal shifts towards encouraging reporting necessitate a reexamination of the demographics and substances involved.

Objectives: This study aimed to investigate demographics and specific drugs in DFSA cases in Harris County, Texas, during 2023. Understanding current trends can inform testing protocols, scope, and improve future interpretation.

Methods: Data from 146 DFSA cases submitted to the Harris County Institute of Forensic Sciences in 2023 were evaluated. Testing followed standard protocols. Alcohol screening used dual column headspace gas chromatography flame ionization detection (HS/GC/FID), while 11 drugs/drug classes were screened via enzyme-linked immunosorbent assay (ELISA). Benzodiazepines and ZZZ drugs were analyzed using liquid chromatography tandem mass spectrometry (LC/MS/MS). All drugs were confirmed using GC/MS/(MS) or LC/MS/MS at, below, or close to the recommended ASB cutoffs.

Results: The majority of cases were still reported by females (93%) compared to males (7%). Most victims identified as White (57%) followed by Black (30%) then Hispanic (8%). Victim ages ranged from 2-67 years, with the majority falling between 19 and 25. 57% of the DFSA cases were positive for one or more substance. Cannabinoids (44%) was the most common substance detected followed by methamphetamine (38%), cocaine (37%), then ethanol (32%). Alprazolam and hydrocodone were the most common benzodiazepine and opioid detected, respectively. GHB and ZZZ drugs were not detected in any case in which they were tested. Notably, stimulants as a group, consisting of methamphetamine, amphetamine, methylenedioxymethamphetamine (MDMA), methylenedioxyamphetamine (MDA), cocaine, cocaethylene, benzoylecgonine, phentermine, and/or bupropion, were detected in 52% of the positive cases. Additionally, amphetamine ELISA results yielded false positives in 24% of the cases sent for amphetamine confirmation. All false positives were urine samples.

Discussion: Race and gender did not reflect Harris County demographics, which consist of Hispanics as the largest group at 44%, followed by White (27%), then Black (19%) and females representing 50% of the population. This may signal a continued resistance tied to cultural dynamics influencing reporting.

Common antihistamines, antidepressants, and barbiturates were not in the scope of DFSA testing; thus, the true prevalence of drug findings is likely higher. However, the overwhelming prevalence of stimulatory drugs requires further investigation. It is unclear if their presence is due to mere prevalence in this population or if these substances are playing an active role in the sexual encounters. Drug user populations are inherently vulnerable to these crimes. Furthermore, chemsex practices initially popular among homosexual men are becoming more popular among heterosexual encounters. Methamphetamine is also used as a form of payment in some sex trafficking crimes. The use of stimulants in sexual assaults may be counterintuitive to traditional thoughts regarding the circumstances in which these crimes occur. However, their presence suggests a possible correlation.

Lastly, amphetamine false positives in urine immunoassays are well known due to their cross-reactivity to structurally-related phenethylamines. The false positive rate between DFSA cases and other antemortem cases should be compared, as the false positives in DFSA cases may signal a possible marker not being tested. The use of liquid chromatography quadrupole time of flight mass spectrometry (LC/QTOF/MS) in DFSA casework could elucidate what specific substances may be responsible for these false positives and whether other stimulatory substances should be included in testing.

P39

Fentanyl concentrations for peripheral blood and brain samples in non-medical postmortem cases

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Introduction: Fentanyl prevalence has increased exponentially since the start of the current epidemic in 2016. In postmortem toxicology, interpretation of fentanyl in death investigation cases is now complicated due to the high concentrations detected in the driving population and the fact that most fentanyl overdose cases are from illicit use; a change from the medical fentanyl overdoses seen in the past. Additionally, postmortem redistribution plays a role in fentanyl blood interpretation; thus, analyzing another matrix can be beneficial in determining the cause of death. Brain tissue is a feasible matrix to quantitate for fentanyl due to the isolated and protected location of the brain compared with other tissues during postmortem decomposition.

Objectives: The objective of this study was to determine peripheral blood (PPH) and brain fentanyl concentrations and to evaluate their ratio for postmortem toxicology cases where there was no indication that fentanyl was administered in a medical setting.

Methods: Each laboratory analyzed their own samples and then the results were combined and reviewed together for a total of 569 cases with peripheral blood and brain data. The quantitative reporting limit for blood and brain from Orange County and Oklahoma was 0.5 ng/mL and 2.5 ng/mL, respectively. Cases were separated into three groups categorized as Low, Medium, and High. The groups were determined based on the peripheral blood concentration of 0.5 to < 10 ng/mL, 10 to 50 ng/mL, and concentration higher than 50 ng/mL. The ratio of peripheral blood and brain homogenate was calculated.

Results: The results for the three concentration groups can be seen in Table 1. For all cases, 84% of the decedents were male with the median, average and age range of 34, 37 and 3 – 73 years.

Table 1: Peripheral blood, Brain and PPH:Brain Data for Three Concentration Groupings

	N	PPH Median (Mean, Range) (ng/mL)	Brain Median (Mean, Range) (ng/g)	Ratio Median (Mean, Range)
Low (0.5 - <10 ng/mL)	292	5.2 (5.3, 0.53 – 10)	26 (29, 0.72 – 130)	0.23 (0.30, 0.02 – 2.6)
Medium (10 – 50 ng/mL)	237	9.7 (18, 10 – 50)	34 (53, 6.9 – 680)	0.32 (0.47, 0.05 – 2.8)
High (>50 ng/mL)	40	82 (150, 53 – 1100)	190 (330, 3 – 3100)	0.52 (2.3, 0.07 – 36)
All Cases	569	9.7 (21, 0.53 – 1100)	33 (60, 0.73 – 3100)	0.32 (0.51, 0.02 – 36)

Discussion: Not surprisingly, the five most commonly detected drugs in conjunction with fentanyl were 4-ANPP (42%), amphetamine and/or methamphetamine (23%), naloxone (17%), alprazolam (14%) and benzoylecgonine (8.4%), with stimulants as the most common drug class reported for both laboratories. Both laboratories qualitatively analyze for

norfentanyl in blood and it was detected in 46% of the cases. For all concentration groups, the brain concentration was higher than the blood concentration for the majority of the cases. The median PPH:Brain ratio increased as blood concentrations increased among the groups with the median ratio doubling between the low and high groups. This compilation of fentanyl peripheral blood and brain concentrations will serve as a resource to postmortem forensic laboratories that have only brain specimen for analysis or who have low peripheral blood fentanyl concentrations and need a reference for corresponding brain concentrations.

P40

Detection of Forensically Relevant Nitazenes Using Liquid Chromatography Tandem Mass Spectrometry (LC-MS/MS)

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Introduction: The opioid epidemic remains one of the largest issues facing the United States today, placing associated demands for testing on the forensic science community. Although illicit fentanyl is one of the main contributors to this problem, other novel synthetic opioids (NSO) also play a role. One class of NSO that have become prevalent in recent years are the nitazenes (2-benzylbenzimidazoles). As the number of relevant nitazenes increases, there is a need to continue to develop new methods that contain a variety of structurally diverse compounds including ring-substituted nitazenes and analogs such as 5-methyl etodesnitazene. Additionally, methods such as liquid chromatography tandem mass spectrometry (LC-MS/MS) are becoming increasingly common in toxicology labs so new methods for NSO detection should take advantage of this sensitive instrumentation if they will be integrated into standard workflows.

Objectives: The purpose of this project was to develop and validate a quantitative method for seven target nitazenes (4'-OH nitazene, 5-methyl etodesnitazene, isotonitazene, metodesnitazene, N-piperidinyl etonitazene, N-pyrrolidino etonitazene, and protonitazene) in whole blood using LC-MS/MS.

Methods: Analysis was completed with an Agilent 1290 Infinity II Liquid Chromatograph and an Agilent 6470 Triple Quadrupole Mass Spectrometer with positive electrospray ionization operated in multiple reaction monitoring mode. The column was an Agilent Poroshell 120 EC-C18 column (2.1x100mmx2.7µm) and matching guard column. Gradient elution (0.4 mL/min) was employed with 5 mM ammonium formate and 0.1% formic acid in deionized water and 0.1% formic acid in acetonitrile as mobile phases A and B, respectively. The total run time was nine minutes. A solid-phase extraction protocol was developed using Cerex Clin II cartridges with four wash steps and elution with 95:5 ethyl acetate: ammonium hydroxide; preconditioning was not required. Isotonitazene-$^{13}C_6$ was used as an internal standard (5 ng/mL in 0.5 mL blood); metodesnitazene-D_4 was also investigated. Matrix effects, calibration model, bias, precision, limit of detection (LOD), limit of quantitation (LLOQ), carryover, and interferences were evaluated based on ANSI/ASB 036. Additional matrix effects experiments were completed with three human and three bovine sources to verify method performance between matrices.

Results: Validation criteria were met as described in ANSI/ASB 036. Calibration curves were constructed with 8 non-zero calibrators (0.5–100 ng/mL). A 1/x-weighted quadratic model was selected for 5-methyl etodesnitazene while a 1/x-weighted linear model was selected for the remaining analytes. LOD criteria were met at 0.5 ng/mL for 5-methyl etodesnitazene, 4-OH nitazene, N-piperidinyl etonitazene, and N-pyrrolidino etonitazene and at 0.25 ng/mL for other analytes. Bovine and human sources were comparable and matrix effects were within ±25% (±15% CV) for all analytes and isotonitazene-$^{13}C_6$. Higher suppression (-30%) was observed with metodesnitazene-$D_{4;}$ %CV was within 15%. Therefore, matrix effects were considered acceptable and additional matrix sources were used in validation to verify performance at the LOD and LLOQ. Grand bias was between -19% and +4% and precision was within 17% across analytes and concentrations. The greatest bias was observed with metodesnitazene and 5-methyl etodesnitazene at lower concentrations. No interference was observed between analytes, internal standards, or blank matrices. Carryover was well below the LOD.

Discussion: An LC-MS/MS method was developed and validated for detecting several nitazenes in whole blood at forensically relevant concentrations. Comparison with human blood supports the use of bovine blood to prepare calibrators and controls. The working range of 0.5–100 ng/mL is appropriate for application based on previously published methods, and the method's performance was validated for this range with LODs ranging from 0.25–0.5 ng/mL in blood. This is a sensitive method for quantitating seven prevalent nitazenes using analytical conditions and reagents commonly used in forensic laboratories.

P41

Qualitative Detection of Novel Urine Synthetic Cannabinoids and Positivity Rates

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Introduction: Synthetic cannabinoids (SCs) are psychoactive substances designed to mimic the effects of Δ9-tetrahydrocannabinol (THC). However, many SCs exhibit higher binding affinities to cannabinoid receptors 1 and 2 (CB1 and CB2) compared to THC. Beyond binding to these receptors, SCs also interact with non-cannabinoid targets, resulting in complex pharmacological effects and diverse toxicity profiles. Due to their hydrophobic nature, extensive metabolism, and continuously emerging novel SCs, these compounds are challenging to detect in urine, leading to false-negative results on common drug screens used in hospitals and pre-employment screening programs.

The laboratory developed and validated an automated screening method for the qualitative liquid chromatography tandem mass spectrometry (LC-MS/MS) detection of 39 novel SCs in human urine following a 15-minute room temperature enzymatic hydrolysis. The qualitative LC-MS/MS screening method was validated using 3 non-zero calibrators and has been utilized to analyze 7123 specimens from court-ordered drug monitoring and drug rehabilitation programs. The new method automated a dilute-and-shoot preparation utilizing room temperature hydrolysis and MRM (multiple reaction monitoring) analysis on a 6500+ MS/MS. The analyte list was updated based on current trend reports from the Center for Forensic Science Research and Education (CSFRE) and College of American Pathologists (CAP).

Objectives: The attendee will learn about the laboratory's latest novel qualitative screening method for the detection of SCs in urine and the positivity rates from these specimens sourced from court ordered drug monitoring and drug rehabilitation programs.

Methods: Method validation studies included accuracy, precision, LOD/LLOQ (limit of detection/lower limit of quantitation), carryover, matrix effects, stability, and interference from over 100 related and non-related drugs. Specimen preparation involved incubating 50 µL of the sample in 150 µL of IMCS RT enzyme-internal standard mix for 15 minutes at room temperature. The solution was mixed with a 50:50 solution of 0.1% formic acid with 2 mM ammonium formate in deionized water to 0.1% formic acid with 2 mM ammonium formate in acetonitrile. The sample preparation, incubation, extraction and final vortex step were performed on the Hamilton® Nimbus platform. Samples were subsequently centrifuged at 1570 G and 24^{0}C for 20 minutes and transferred to LC-MS/MS for analysis. Analysis was performed on an Agilent 1290 Infinity II LC coupled to a Triple Quad 6500+ MS/MS (SCIEX) by injecting 20 µl on to a Raptor Biphenyl, 2.7 µm, 50 x 4.6 mm column. Gradient elution using A) 0.1% formic acid in deionized water and B) 0.1% formic acid in methanol was conducted and MRM data were acquired over a total run time of 6 minutes.

Results: Reporting cutoffs were 5 ng/mL on a 3-point calibration curve (5, 10 & 20 ng/mL) with a linear $1/x^2$ regression, producing $r \geq 0.98$ for all analytes. LLODs were 2.5 ng/ml for all analytes except 4-F-MDMB-BUTINACA butanoic acid MTB, and 5-F-EDMB-PINACA (5 ng/mL). With an acceptability criterion of ≤20%, the inter and intra-day precision (CV) for all analytes were satisfactory at <11%. No carryover was observed for any analyte at concentrations up to 500 ng/mL. No interferences were observed from any of the over 100 related and non-related drugs tested. Ionization enhancement/suppression at reporting cutoffs was acceptable and prepared specimens were stable for 72 hours in the instrument autosampler at 15°C.

Over the months of March 2024-May 2024, a total of 7123 samples were tested with an overall positivity of 19% recorded. The latter refers to the proportion of samples positive for at least one analyte relative to the sum total of samples tested. MDMB-4en-PINACA and ADB BUTINACA N-(4-hydroxybutyl) metabolites exhibited the highest prevalence rates at 9.96 % and 3.37 %, respectively. These prevalence rates were calculated relative to the sum total of samples tested.

Conclusion/Discussion: Development and validation of an automated dilute-and-shoot method for the simultaneous qualitative LC-MS/MS analysis of 39 SCs in urine has been completed in our privately owned laboratory. Reliable qualita-

tive detection was validated with a 5 ng/mL cutoff. Prevalence from 3 months testing was also presented.

P42

Postmortem Distribution of Fentanyl and its Metabolites

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Introduction: In 2023, fentanyl was reported to be responsible for approximately 70% of all overdose-related deaths in the United States. These overdose-related deaths stem from fentanyl's high potency. While blood fentanyl concentrations can aid in case interpretation regarding potential overdose-related deaths, blood is not always available for toxicological testing. Therefore, research needs to be conducted to determine if fentanyl concentrations observed in alternative matrices can be correlated with postmortem blood concentrations.

Objectives: To determine the distribution of fentanyl, norfentanyl, and 4-anilino-N-phenethyl-4-piperidine (4-ANPP) in samples collected from aviation accident victims and examine how blood concentrations correlate with those observed in alternative matrices.

Methods: A 'crash-and-shoot' extraction followed by a UPLC/MS/MS method was developed and validated for the quantitative analysis of fentanyl, norfentanyl, and 4-ANPP in various biological matrices, including blood, urine, serum, brain, liver, lung, and kidney. Following method validation, samples from 18 previously analyzed fentanyl-positive cases were reanalyzed to compare the concentration of fentanyl, norfentanyl, and 4-ANPP in alternative matrices with those found in blood. Matrices analyzed included: bile (n=9), blood (n=14), brain (n=13), cerebral spinal fluid (n=3), heart (n=13), kidney (n=12), liver (n=13), lung (n=14), muscle (n=14), plasma (n=1), serum (n=2), spleen (n=13), urine (n=8), and vitreous humor (n=7). Alternative matrices with matrix:blood concentration ratios that had CVs of less than 25% were deemed to correlate and those matrices could be cautiously used to estimate a postmortem blood concentration in the absence of blood.

Results: The validated method consisted of sampling 0.5 mL or 0.5 g of specimen, adding 25 µL of a 500 ng/mL isotopically labeled internal standard mixture (12.5 ng), crashing with 2 mL of ice-cold methanol, vortexing briefly, and centrifuging the sample for 10 minutes at 1500 x g. After centrifugation, 300 µL of sample was transferred to a 0.2 µm PVDF Thomson filter vial. The method had a calibration range of 0.05 – 50 ng/mL. Other validation tests performed included limit of detection, bias and precision, stability (post-extraction, refrigerator, and freeze/thaw cycles), matrix effects, recovery, process efficiency, tissue control bias and precision, dilution integrity, carryover, interference, and cross contribution.

The distribution study did not find any correlation between the concentration of fentanyl, norfentanyl, or 4-ANPP in alternative matrices when compared to blood. For fentanyl, the largest alternative matrix:blood ratio was observed in urine (12.2 ± 24.7). For norfentanyl, the largest alternative matrix:blood ratio was also observed in urine (17.3 ± 44.2). For 4-ANPP, the largest alternative matrix:blood ratio was observed in liver (26.2 ± 23.8). For all matrices but urine and vitreous humor, there was an average norfentanyl:fentanyl ratio of <1.0; urine had an average ratio of 5.5 and vitreous humor had an average ratio of 1.6. For all matrices, there was an average 4-ANPP:fentanyl ratio of <0.05. Due to the low number of replicates, comparisons to blood were not made for plasma (n=1) nor serum (n=2). Additionally, two cases were excluded from the final distribution results as all fentanyl, norfentanyl, and 4-ANPP results were found to be below the methods limit of detection; these cases had fentanyl concentrations near the limit of detection and undetectable norfentanyl concentrations during the initial analysis performed for casework.

Discussion: This study outlines a validated 'crash-and-shoot' method followed by UPLC/MS/MS analysis for fentanyl, norfentanyl, and 4-ANPP in biological matrices. The method proved to be robust and sensitive, having a lower limit of quantitation of 0.05 ng/mL for all three analytes. A postmortem distribution study was performed to examine the relationship between fentanyl, norfentanyl, and 4-ANPP concentrations in alternative matrices to that of blood. No reliable

correlation was observed, suggesting concentrations of these drugs found in postmortem alternative matrices cannot be used to estimate a postmortem blood concentration.

P43

Novel LC-MS/MS Screen of Designer Stimulants in Urine Method

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Introduction: Synthetic stimulants, such as cathinones, have been developed as recreational drugs with psychoactive properties. Synthetic cathinones, colloquially known as "bath salts," are chemically related to the naturally occurring stimulant cathinone, found in the khat plant. These substances are designed to mimic the effects of traditional stimulants such as amphetamines and cocaine, often resulting heightened euphoria, increased energy, and enhanced sociability. However, they also carry severe adverse effects, including paranoia, hallucinations, cardiovascular complications, and in some cases death. Two emerging designer stimulants are α-pyrrolidinohexanophenone (α-PHP) and α-pyrrolidinoisohexanophenone (α-PiHP) that are derivatives of the pyrovalerone subgroup and are positional isomers of each other. Dipentylone, another synthetic cathinone, features a methylenedioxy ring, similar to MDMA, and induces stimulant and empathogenic effects.

Designer stimulants are excreted in urine unchanged and as metabolites. In the case of α-PHP and α-PiHP, the primary metabolic pathway primarily undergoes hydroxylation, oxidation, and N-dealkylation, while dipentylone is metabolized through reduction of the beta-keto group forming norephedrine and norpseudoephedrine analogs, as well as hydroxylation and demethylation. In this study, we focus on the parent drugs, α-PHP and α-PiHP, and the metabolite pentylone.

Objectives: Attendees will learn about Abbott Redwood Toxicology Lab's latest novel method for qualitatively confirming eighty-nine drugs in urine via liquid chromatography tandem mass spectrometry (LC-MS/MS) and observe the method's 2024 positivity rates. Additionally, the attendees will learn of the prevalence of dipentylone, α-PHP and α-PiHP in urine specimens from court-ordered drug monitoring and drug rehabilitation programs.

Methods: The laboratory developed and fully validated an automated MRM based LC-MS/MS screening method for qualitatively detecting designer stimulants, amphetamines, fentanyl, opiates, benzodiazepines, TCAs, and SSRIs in urine following ANSI/ASB Standard 036. The validation process tested parameters including linearity, precision, ionization, interference, and LC carryover. Urine specimens are mixed with a methanol and deuterated internal standard/ICMS enzyme mix, incubated for 15 minutes at room temperature. After hydrolysis, 300 µL 50/50 Acetonitrile/Water containing 0.1% formic acid and 2 mM ammonium formate is added and centrifuged at room temperature, 1570 G for 20 minutes. Subsequently, 200 µL of supernatant is diluted in 200 µL of 2 mM ammonium formate with 0.1% formic acid in deionized water before 10 µL is injected onto an Agilent 1290 Infinity II using a Raptor Biphenyl 2.7 µm, 50 x 4.6 mm column with a Restek Ultrashield UHPLC 0.2 µm precolumn filter with detection on a 6500+ Triple Quad mass spectrometer (AB SCIEX) with a total run time of 9.5 minutes. The mobile phase A consists of 0.1% formic acid and 2 mM ammonium formate in deionized water, and mobile phase B consists of 0.1% formic acid and 2 mM ammonium formate in 98:2 acetonitrile: deionized water.

Results: The method launched on February 5, 2024, and the laboratory operations has tested a total number of 2486 samples in the first 17 weeks. The positivity rate in the order of prevalence is dipentylone/pentylone (3.66%), α-PiHP (2.05%), and α-PHP (0.12%).

Imprecision at the lowest calibrator (5 ng/mL) ranged from 5.4% - 7.2%. A LLOD was established at 5 ng/mL for dipentylone/pentylone, α-PHP, and α-PiHP. No interferences were observed from any of the 100 related and non-related drugs tested. Ionization enhancement/suppression at reporting cutoffs was acceptable and prepared specimens were stable in the autosampler at 15 °C for 72 hours. No LC-MS/MS carryover was observed at 500 ng/mL.

Discussion: After launching this assay, we observed that specimens positive for α-PiHP occasionally screened positive for phencyclidine (PCP) using our DRI homogeneous enzyme immunoassay. However, these specimens did not confirm as containing PCP. To investigate this, we prepared varying concentrations of α-PiHP and found that concentrations above 500 ng/mL resulted in positive PCP screens, indicating cross-reactivity or interference.

This method offers high throughput and significantly shorter turnaround times, attributed to the combined nature of the assay. Additionally, the data highlights the prevalence of these analytes, expanding awareness in the forensic community.

P44

Drugs of abuse detected in oral fluid specimens of drivers in the state of Victoria (Australia) from 2008-2022

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Introduction: Australian Roadside Drug Testing (RDT) programs aim to deter drug-impaired driving via high-visibility and high-volume roadside-screening of oral fluid (OF) combined with laboratory confirmation.

In December 2004, Victoria commenced a world first OF RDT program targeting methylamphetamine (MA), 3,4-methylenedioxy-N-methylamphetamine (MDMA) and delta-9-tetrahydrocannabinol (THC) under the Road Safety Act (RSA) of the State Government of Victoria, Australia. This program uses Securetec DrugWipe®, OF swab. If a driver tests positive at the roadside, an OF specimen is collected and sent to the Victorian Institute of Forensic Medicine (VIFM) toxicology laboratory for confirmatory analysis using liquid chromatography tandem mass spectrometry.

Objectives: In addition to the 3 RSA drugs, as part of the confirmatory analysis the specimens were also monitored for 40 additional drugs, including opiates/opioids, stimulants, benzodiazepines, ketamine and cannabinoids. The presented retrospective study aimed to determine the prevalence and any trends in the detection of these additional drugs over the last 15 years (2008-2022).

Methods: Positive confirmatory detections for RSA drugs were reported if acceptance criteria for identification were met and concentrations were above the confirmatory test cut-off, as specified in the Australian standard AS4760:2006. Analytical data files (n= 89,744) from driver OF samples submitted for confirmatory analysis between 2008–2022 were retrospectively interrogated for additional drugs. The results were collated into annual batches and processed in a single results table for each drug and metabolite.

Results: At least one RSA drug was detected in 93.6% of the submitted OF specimens, with MA being the most common at 74.8%, followed by THC at 33.9% and MDMA at 5.2%. Other drug classes frequently detected were opiates/opioids, cocaine and metabolites, and benzodiazepines in 12%, 6.3% and 5.2% of cases respectively. Specimens in which opiates/opioids were detected, 4.2% were positive for 6-monoacetylmorphine (6AM) and 3.2% for methadone or EDDP and were most prevalent in the drivers that were confirmed positive for MA. The highest percentage of cocaine and cocaine metabolites were detected in 2009 (10.4%), and the lowest in 2014 (3.5%). Since 2014, the percentage of cocaine-positive specimens steadily increased, peaking in 2019 (7.9%), and subsequently has declined annually. In cases confirmed with MDMA and no other RSA drugs, cocaine, or cocaine metabolites, were detected in 40.4% of cases and, ketamine was detected in 35.2%. This is in striking comparison to cases confirmed with MA positive and no other RSA drugs, where cocaine, or cocaine metabolite, were detected in 4.4% and ketamine 0.6%. Likewise, confirmed THC positive cases with no other RSA drugs, cocaine, or cocaine metabolites, were detected in similar proportions, 5.0%, and ketamine 0.8%. Over the 15-year period the majority (74%) of the benzodiazepines positive cases were due to the presence of diazepam.

Discussion: While the incidences of RSA drugs alone are concerning, it is evident many drivers are also concomitantly using other potentially impairing drugs. Detectable levels of illicit drugs in OF specimens above a cut-off, such as RSA drugs, and potentially 6AM or cocaine may be used to provide an indication of relatively recent drug exposure and, therefore higher risk of potential impairment. However, detecting and legislating against misuse of prescription drugs, such as benzodiazepines and certain opioids/opiates in OF specimens, remains impractical. It is acknowledged that studying this cohort of the OF RDT driving population in Victoria has inherent limitations (polydrug use), and findings should not be viewed in isolation when trying to understand the prevalence of other drugs present in the general driving population. However, this data provides epidemiological evidence that may be of interest to inform road safety initiatives.

P45

Triggered multiple reaction monitoring LC-MS/MS method for screening of 90 drugs of abuse (including NPS) in hair

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Introduction: In Singapore, individuals who have completed drug abuse treatment undergo regular screening to prevent relapse. Hair testing is preferred over urine testing due to its longer detection window, allowing for less frequent and less disruptive testing, which supports better reintegration into society. With the increasing abuse of new psychoactive substances (NPS), the laboratory requires a comprehensive screening method capable of analysing conventional drugs and NPS concurrently in hair, with the ability to expand the testing scope to accommodate emerging NPS.

Objectives: A LC-MS/MS method for screening of 90 drugs of abuse, including conventional drugs, synthetic cathinones, synthetic cannabinoids, LSD, fentanyl, mitragynine in hair was developed and validated.

Methods: 20 mg of hair sample, that has been washed with methanol, was pulverized in 1 mL of pH 6 sodium phosphate buffer before undergoing extraction using a Chromabond® Drug SPE cartridge. The cartridge was washed with deionised water and 0.1M acetic acid, followed by elution with methanol: ammonia (98:2 v/v). The eluate was then treated with 0.12% HCl in methanol, dried down under a stream of nitrogen at 40°C, and reconstituted with 100 µL of water: acetonitrile (50:50 v/v).

Chromatographic separation was achieved on a Kinetex Biphenyl column (2.1 x 100 mm, 2.6 µm) with gradient elution using solvent (A) 10 mM ammonium formate (0.1% formic acid) and solvent (B) acetonitrile (0.1% formic acid), at a flow rate of 0.4 mL/min, with a total run time of 13 minutes.

Mass spectrometric data was acquired in triggered multiple reaction monitoring (tMRM) mode with positive electrospray ionization using an Agilent G6495C iFunnel Triple Quadrupole LC/MS. In tMRM mode, a primary transition for quantitation and a series of secondary transitions for identification are acquired for each analyte, which can be combined into a product ion spectrum for library matching. The acquisition of secondary transitions is triggered when the primary transition exceeds the set abundance threshold, allowing for expansion of analyte list without compromising sensitivity.

Results: The limits of detection were determined to be 10 to 15 pg/mg for conventional drugs and 1 to 20 pg/mg for NPS and their metabolites. Extraction recoveries ranged from 40% - 76% for conventional drugs and 10% to 96% for NPS. Synthetic cannabinoids metabolites such as MDMB-4en-PINACA ester hydrolysis dihydrodiol metabolite had particularly low recoveries due to the acidic nature of the analytes. Despite the low recoveries observed for acidic analytes, the desired limit of detection was achieved.

The intra-day and inter-day precisions for all analytes ranged from 0.9% to 26.6% and 0.8% to 13.9%, respectively, with bias ranged from -28.3% to 18.8%. No interference was observed from common drugs of abuse and structurally similar analytes, except for phentermine, which shares the same primary transition and elutes closely to methamphetamine's peak. However, phentermine and methamphetamine can be distinguished from their product ion spectrums. No significant endogenous interference was observed. Extracted samples were found to be stable for at least 3 days when kept at 4°C and 15°C.

Discussion: The validated method was successfully applied on 40 authentic hair samples from drug abusers between 2021 and 2022. Among these samples, methamphetamine was the most commonly detected conventional drug detected, found in 31 samples, followed by MDMA, which was detected in 15 hair samples. Out of the 15 hair samples that tested positive for MDMA, 10 also found to contain ketamine.

MDMB-4en-PINACA was the most common NPS detected in hair samples, followed by BZP and 4-FPP. BZO-HEXOXIZID was detected in one of the hair samples analysed. Most of the hair samples tested positive for NPS also contained conventional drugs, particularly methamphetamine and MDMA.

P46

The Development of a Hair Inventory Tool and its Importance to Drug Testing in Hair

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Introduction: Drug testing in hair has an extended window of detection which can average 1-month per half inch of hair, suggesting that a 1.5-inch section of hair records approximately 90-days of drug use making hair a desirable matrix for testing donors who use drugs intermittently. Although hair testing is an attractive addition to traditional drug testing matrices (e.g., urine, blood, oral fluid, etc.) it faces challenges related to the categorization of donor hair attributes. The National Laboratory Certification Program (NLCP) has developed a Hair Inventory Tool for comprehensive cataloging of hair samples for research and proficiency testing.

Objectives: To develop a web-based tool to inventory all hair samples in the NLCP library and allow queries of various attributes to increase efficiency in identifying specimens for both testing and research purposes.

Methods: All available donor data (i.e., gender, age, race, drug history, etc.) was cataloged into applicable data fields for the hair samples. After visual inspection, subjective and objective attributes were entered into standardized fields for data consistency and analysis (i.e., hair color, curl pattern, sample condition, condition of hair, length of hair).

Hair color was evaluated based on the International Hair Colour Level System (Schwarzkopf). The curl pattern was defined as straight, wavy, or coily. Sample condition was described as either intact or damaged. Objective measurements included form of hair (i.e., strands, snippets, pulverized) and length of hair.

The data entry fields were used to build relational tables capable of data entry and data searching. The web application can track hair specimens used in performance testing (PT) production or applied studies and identify samples for future activity.

Fields to capture quantitative drug testing results reported by the reference testing laboratory are also included and are linked to the hair samples by a unique sample ID auto-generated once the data entry is complete.

Results: All hair in NLCP inventory was cataloged into the web-based tool. Reference lab quantitative results for drug analytes uploaded included quantitative analyte concentrations (pg/mg) and qualitative results (positive or negative). No confirmation cutoffs were applied so specimens with any amount of drug analyte present above the limit of quantitation of the assay were qualitatively identified as positive.

Currently there are 590 hair samples logged in NLCP inventory. Samples colored light brown and dark blonde are most prevalent. Of the 281 specimens identified as positive, cocaine and its metabolite, benzoylecgonine, are most prevalent, followed by samples positive for Δ-9-tetrahydrocannabinol-9-carboxylic acid.

Discussion: The web tool catalogs samples in NLCP inventory, and the application dashboard details the total number of samples with an overall specimen disposition of positive or negative, the number of samples in each color category (Schwarzkopf scale), and the number of samples positive for various drug analytes.

The tool dashboard tracks inventory levels indicating shortages of hair samples with various attributes of interest (ex: light blonde) or drug dispositions. Procurement of commercial hair samples and targeted collections from substance abuse treatment facilities can fill in gaps where there is lack of inventory of hair samples with desired attributes.

The NLCP hair library supports hair research by providing tailored sample sets including specimens positive for specific drugs and/or meeting specific criteria regarding hair color, length, curl pattern and condition. Given the complexity of drug testing in hair, authentic hair specimens are often essential to conduct impactful and scientific studies. The NLCP is

interested in collaborations with other researchers studying topics relevant to workplace drug testing in hair.

P47

Non-selective Sample Preparation Approaches Using Extrahera® Automation for Broad-spectrum DoA Whole Blood Analysis

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Introduction: Forensic and toxicology laboratories conduct broad-spectrum whole-blood (WB) screening to detect potential drug exposures. These assays target dozens to hundreds of analytes simultaneously, including prescribed medications, illicit drugs, and over-the-counter medications, as well as their metabolites. However, extracting a wide range of drugs of abuse (DoA) analytes from WB can be challenging due to the complex nature of the sample matrix. While protein precipitation is a simple method, it does not provide thorough matrix removal for WB. Selective techniques like solid phase extraction (SPE) offer better sample cleanup; however, the specificity of the interaction makes it difficult to balance the extraction efficiency and sample cleanliness. The nonselective techniques explored here can effectively remove the interference components (e.g., proteins, phospholipids) without compromising the extraction recovery for a wide range of analytes.

Objectives: This presentation demonstrates an automated sample preparation workflow for the broad-spectrum DoA WB analysis using two non-selective sorbent chemistries: protein and phospholipid depletion (PLD) and supported liquid extraction (SLE).

Methods: A panel of 58 analytes with a diverse chemical backbone and a wide range of LogP (-0.6-- 7.05) and pKa (0.13-10.47) was spiked into the donor WB sample to evaluate the method's feasibility. The Extrahera® sample preparation workstation automatically extracted samples using the Biotage ISOLUTE® PLD+ and SLE+ plates. Samples were also processed by the protein precipitation and SPE with different sorbent chemistries (e.g., ABN, CX, and WCX) for comparison. Extraction performance was evaluated by extract recovery, matrix effect, and reproducibility in low (5 ng/ml) and high (50 ng/ml) concentrations.

Results: The PLD method demonstrated excellent extraction performance for diverse analytes, with high recovery (70%-90%), minimal matrix effect (0.9-1.2; where <1 indicates ion suppression), and good reproducibility (RSD, 6-15%). SLE method achieved high extraction recovery (70%-80%) for analytes more soluble in organic solvents over aqueous with superior matrix effect (0.95-1.0) and reproducibility (RSD, <10%). Phospholipid removal was determined by monitoring several common phospholipid transitions (e.g. 184-->184) and both PLD and SLE methods removed >99% of the phospholipids compared to standard protein precipitation methods (protein crash), effectively improving ion suppression. In contrast, when considering the extraction recovery across a wide range of analytes, the SPE methods only removed about 50% of the phospholipids and was effective for a limited set of analytes with similar retention behaviors on the sorbent. The sample extraction time for each 96-well PLD or SLE plate was around 50-55 min, while it took at least 70 min when using SPE methods due to the needed wash steps. The automized sample preparation workflow offered better reproducibility than manual methods and greatly reduced the time scientists spent on bench operation.

Discussion: The non-selective sample preparation techniques, PLD and SLE, are more suitable for broad-spectrum DoA WB analysis than the SPE methods. While SPE methods are highly selective for specific analytes, PLD and SLE focus on removing biomatrix components without being analyte-specific. The PLD method is advantageous in standardized and universal procedures and offers better recovery regardless of structure diversity, while SLE offers superior sample cleanliness and reproducibility. Implementation of laboratory automation through the Extrahera® sample preparation workstation significantly reduces the time spent on bench operations, allowing scientists to allocate more time to engage in creative work.

P48

Stimulants and Hallucinogens – Expanding efficiency and scope using Liquid Chromatography-tandem Mass Spectrometry

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Introduction: Stimulants and hallucinogens are prevalent drugs detected in drug-impaired driving cases throughout the state of Wisconsin. Stimulants and hallucinogens of interest at the Wisconsin State Crime Laboratories (WSCL) include, but are not limited to, ecgonine methyl ester, cocaine, cocaethylene, benzoylecgonine, amphetamine, methamphetamine, MDMA, PCP, and ketamine. Historically, four different analyses were required to detect these major drugs and their metabolites. These previous methods utilized solid-phase and liquid-liquid extractions using 250-1000 µL of sample for each analysis. Using Liquid Chromatography-tandem Mass Spectrometry (LC/MS/MS), these four methods can be combined and limit resources. This method also allows for increased sensitivity and detection of Novel Psychoactive Substances (NPS) within these drug classes as these were not previously detectable with Gas Chromatography-Mass Spectrometry (GC/MS).

Objectives: The objective of this method validation is to increase productivity through the combination of methods, increase scope through the addition of NPS drugs, and lower detection limits of stimulants and hallucinogens. Analysis of this method on LC/MS/MS rather than GC/MS also decreases the run time which increases efficiency and reduces resources within the laboratory.

Methods: This method was validated to ANSI/ASB Standard 036, Standard Practices for Method Validation in Forensic Toxicology (1st edition, 2019). Blood was validated for quantitative analysis while urine, plasma, and serum were validated for qualitative analysis. This method requires 250 µL of sample which is then manually extracted via solid-phase extraction (SPE). United Chemical Technologies SPE 96-well plate – Long Drip Tip, Cleanscreen® Extraction Column were utilized for this extraction to increase efficiency while reducing resources and consumables. Extracted samples were then analyzed via a Waters ACQUITY UPLC H-Class/Xevo TQD instrument utilizing an ACQUITY Ultra-Performance Liquid Chromatography BEH C18 column (2.1 mm*100 mm, 1.7 µm) for separation and electrospray ionization in positive ion mode for detection.

Results: All criteria of ANSI/ASB Standard 036 were met during this method validation. As a result, ecgonine methyl ester, cocaine, cocaethylene, benzoylecgonine, amphetamine, methamphetamine, phentermine, MDA, MDMA, MDEA, ketamine, norketamine, and PCP may be quantitated in blood and qualitatively confirmed in urine, plasma, and serum. Additionally, ephedrine/pseudoephedrine, norephedrine, norpseudoephedrine, cathinone, N-ethylamphetamine, 2-fluoro-deschloroketamine, deschloroketamine, fluoroexetamine, 3/4-OH-PCP, 3/4-MeO-PCP, dimethyl pentylone, pentylone, eutylone, N-cyclohexyl methylone, a-PHP/PiHP, MDPHP, N-cyclohexyl butylone, N-propyl butylone, 2-MMC, 3-MMC, mephedrone, MDPV, methylone, methedrone, methylphenidate, atomoxetine, phenethylamine, phenmetrazine, bupropion, and OH-bupropion were validated for qualitative confirmation in blood, urine, plasma, and serum. Analytes with slashes were not able to be separated within the LC/MS/MS analysis and will therefore be reported as both isomers.

Drug or Metabolite	Working Range (µg/L)	LLOQ	High Control (%bias)	Mid Control (%bias)	Low Control (%bias)
Ecgonine Methyl Ester	5-500	5	0.99	-3.08	-4.86
Cocaine			0.86	4.00	3.17
Cocaethylene			5.18	2.30	-2.04
Benzoylecgonine	20-1500	20	5.54	-2.31	-0.53
Amphetamine	10-1000	10	-4.77	1.73	-1.76
Methamphetamine			2.41	5.24	0.66
Phentermine			-0.11	1.02	-2.19
MDA			-0.56	5.66	-3.50
MDMA			5.72	4.04	-0.25
MDEA			-3.46	2.31	-0.40
Ketamine			-0.98	-0.68	1.36
Norketamine			0.10	4.03	-3.08
PCP	10-500	10	-1.43	0.72	-1.33

Discussion: This newly validated method has successfully created a more efficient extraction when it comes to resources such as time, sample volume, and consumables. Stimulants and hallucinogens are now extracted and processed faster with the combination of techniques and the use of SPE and LC/MS/MS methods. The confirmation and quantitation, when applicable, of 47 stimulants and hallucinogens has successfully expanded the scope and detection of the WSCL's methods with the possibility of adding additional analytes in the future.

P49

Identification of Bongkrekic Acid in Postmortem Samples from a Foodborne Poisoning Incident in Taiwan

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Introduction: Foodborne poisoning poses significant public health risks. Bongkrekic acid (BKA), a rare mitochondrial toxin produced by *Burkholderia gladioli pathovar cocovenenans*, has been implicated in several fatal food poisoning incidents in the world, predominantly linked to fermented food products. This study examines a fatal foodborne poisoning incident in Taiwan in March 2024. Identifying the toxic agent in postmortem samples is crucial for confirming the cause of death and preventing future outbreaks.

Objectives: The primary objective was to identify and confirm the presence of BKA in postmortem samples from the victims of a foodborne poisoning incident in Taiwan. We aimed to establish a reliable analytical method for detecting BKA in various biological samples, facilitating future toxicological investigations.

Methods: Postmortem samples, including blood, gastric juice, liver, kidney, heart, spleen, lung, and brain were collected from two deceased males. Samples underwent initial preparation involving homogenization and protein precipitation with organic solvents. An ultrahigh-performance liquid chromatography (UPLC) system paired with a high-resolution time-of-flight mass spectrometer (TOF-MS) was used to identify BKA in the forensic specimens. Identification was achieved by comparing the accurate mass, retention time, and MS^2 spectra with those of an authentic BKA standard. An independent UPLC method coupled to a triple quadrupole mass spectrometer (QqQ-MS) with multiple reaction monitoring (MRM) mode was used to confirm and quantify the BKA levels. Calibration curves were generated using standards of BKA to ensure accuracy and precision. Method validation, including assessments of sensitivity, linearity, precision, and accuracy, was performed to ensure the reliability of the method.

Results: BKA was detected in postmortem blood and gastric contents using UPLC TOF-MS with matched retention times, MS^2 spectra, and a ppm error of less than 5. An independent LC gradient with QqQ-MS confirmed the identification. A 6-minute LC gradient was optimized for MRM transitions for BKA. The analytical range for blood was 5-500 ng/mL with a coefficient of determination greater than 0.995. Precision at three levels was within 15% coefficient of variation (CV), and accuracy ranged between 80-120%. For solid tissues, a standard addition (SA) approach with at least three calibrators per specimen was used, all with coefficients of determination greater than 0.995. Postmortem blood samples had BKA concentrations of 498.1 and 152.32 ng/mL. The highest tissue concentrations were in the kidney (429.97 and 1434.89 pg/mg) and liver (110.07 and 104.57 pg/mg). These results confirmed BKA as the causative agent in the poisoning incident.

Discussion: Identification of BKA in the postmortem samples aligns with the symptoms and fatalities observed, corroborating its known toxicity. The successful use of UPLC-MS/MS underscores the method's efficacy for toxicological investigations. Our findings emphasize the importance of rapid, accurate detection of foodborne toxins to inform public health responses and prevent further cases. Our research provides a framework for future research on foodborne toxicants and highlights the need for stringent monitoring of fermented food products. Confirmation of BKA in the Taiwan incident solidifies its role in the fatalities and underscores the need for effective analytical techniques in public health and forensic investigations.

Conclusion: Detection of BKA in postmortem samples from the Taiwan poisoning incident confirms its role as the toxic agent responsible for the fatalities. These findings contribute to the understanding of foodborne poisonings and highlight the critical need for effective analytical techniques in public health and forensic investigations.

P50

Consolidating LC-MS/MS Method Conditions for the Analysis of Alcohol Metabolites, Barbiturates, and Drugs of Abuse

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Introduction: Efficiency is key in toxicology laboratories where multiple drug panels must be run daily. Consolidating multiple drug panels into large, multi-class drug assays can streamline the analytical testing process and reduce operating costs. To simplify the analysis of alcohol metabolites, barbiturates, THC metabolites, and drugs of abuse, three different LC-MS/MS methods were developed for each analyte class using the same analytical column and mobile phases.

Objectives: The primary objective is to demonstrate the analysis of alcohol metabolites, drugs of abuse, and barbiturates in urine using one set of LC-MS/MS method conditions.

Methods: A panel of 100 drugs of abuse, novel psychoactive substances, therapeutic drugs and metabolites, a panel of biomarkers of alcohol consumption, and a panel of barbiturates and cannabinoid metabolites were all analyzed using a Force Biphenyl 50 x 3 mm, 3 µm analytical column equipped with a Force Biphenyl EXP guard column and a 0.2 µm UltraShield frit. All method development was performed on a Shimadzu LCMS-8045. Mobile phase A consisted of 0.1% formic acid in water and mobile phase B consisted of 0.1% formic acid in methanol for all methods. The column temperature for all methods was 30°C. The injection volume for all methods was 10 µL. The drugs of abuse method that includes new and emerging novel psychoactive substances utilized gradient conditions with a cycle time of 11 minutes and was acquired in ESI+ mode. Urine samples underwent enzyme hydrolysis to convert glucuronide conjugates to their free forms. Barbiturates and Δ9-THC-COOH were analyzed in ESI- mode and have a total run time of 5 minutes. These compounds were spiked into urine and diluted at a 1:10 ratio with water. Finally, alcohol metabolites were analyzed in ESI- mode with a total analysis time of 5 minutes. These compounds were spiked into urine and diluted at a 1:10 ratio with water.

Results: The biphenyl stationary phase has unique selectivity due to the pi-pi interactions that occur between the phase and most drugs when compared to a routine C18 phase, allowing for improved resolution of isobars. In the ESI+ panel, 21 sets of isobars were present, all of which are partially or fully resolved by the developed method. In the case of α-PHP and α-PiHP, which are structural isomers, partial resolution was achieved which allows labs to identify which isomer is present in their sample, potentially eliminating the need for confirmatory testing. In the alcohol metabolite panel, urinary interferences that are particularly problematic in this type of testing were resolved without the use of buffer or additional mobile phases helping to streamline analytical testing processes.

Discussion: A panel of 100 drugs of abuse, novel psychoactive substances, therapeutic drugs and metabolites, a panel of biomarkers of alcohol consumption, and a panel of barbiturates and cannabinoid metabolites were all analyzed using the same analytical column and mobile phase set up. This work highlights the ability of one LC-MS/MS set up to analyze multiple toxicology panels. This allows for laboratories to simplify testing procedures, save time, and reduce operating costs.

P51

Expanded Cannabinoid Analysis in 1300 Urine Specimens

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Introduction: Toxicological assays have traditionally focused on Δ^9-THC and its primary metabolites. However, Δ^9-THC isomers and analogs have recently emerged as legal alternatives to traditional cannabis products. Laboratories developing analytical techniques to detect these compounds in various biological matrices are hampered by the ever-changing landscape of these emerging compounds. Additionally, complicated chromatographic separation of isomers is necessary for accurate reporting.

Objectives: A validated method was used to analyze 1300 urine specimens that screened presumptively positive for cannabinoids by immunoassay between April 2022 and May 2024. The prevalence of cannabinoids in these specimens and observed trends will be reported.

Methods: A dual enzyme-alkaline hydrolysis with IMCSzyme and 8N potassium hydroxide was utilized prior to a liquid-liquid extraction with 80:20 (v:v) hexane:ethyl acetate. The following cannabinoids were extracted from urine: Δ^8-THCCOOH, Δ^9-THCCOOH, Δ^{10}-THCCOOH, Δ^8-THCVCOOH, Δ^9-THCVCOOH, 9(*R*)-HHC, 9(*S*)-HHC, 11-OH-9(*R*)-HHC, 11-OH-9(*S*)-HHC, 9(*R*)-HHCCOOH, and 9(*S*)-HHCCOOH. Sample extracts were dried down and reconstituted in 50:50 (v:v) 0.1% formic acid in deionized water (mobile phase A) and 0.1% formic acid in 50:50 (v:v) methanol:acetonitrile (mobile phase B). Confirmation was performed on an Agilent 1290 liquid chromatography system equipped with a Phenomenex Kinetex Polar C18 column (100 x 2.1 mm, 2.6 µm) coupled to an Agilent 6460 tandem mass spectrometer. All validation parameters were conducted in accordance with ANSI/ASB Standard 036.

Results: Quantitative reporting acceptance criteria were met for 7 of the 11 analytes: Δ^8-THCCOOH, Δ^9-THCCOOH, Δ^{10}-THCCOOH, 9(*R*)-HHC, 9(*S*)-HHC, 9(*R*)-HHCCOOH, and 9(*S*)-HHCCOOH. The linear dynamic range for these analytes was 5-100 ng/mL. The remaining four analytes produced acceptable data for qualitative confirmation, with Δ^8- and Δ^9-THCVCOOH unable to be separated chromatographically. This method was used to analyze 1300 specimens that screened presumptively positive by immunoassay. Of these, positive confirmation results (n=1131) were obtained for Δ^9-THCCOOH (n=816), Δ^8-THCCOOH (n=512), 9(*S*)-HHCCOOH (n=199), and 9(*R*)-HHCCOOH (n=112). Eighty-one samples contained $\Delta^{8/9}$-THCVCOOH. Interestingly, 77 cases contained Δ^{10}-THCCOOH, but this analyte was always found in combination with at least one other cannabinoid. Additionally, 51 samples contained HHC metabolites and no THCVCOOH or THCCOOH analytes. Only a small handful of these cases (n=21) displayed evidence of 9(*R*)-HHC, 9(*S*)-HHC, 11-OH-9(*R*)-HHC, and/or 11-OH-9(*S*)-HHC.

Discussion: This method demonstrated successful confirmation of 11 cannabinoid compounds in urine specimens. A simple liquid-liquid extraction coupled with a sensitive and robust instrumental method allowed for the confirmation of cannabinoid metabolites, while overcoming the challenges of adequate chromatographic separation for THC and HHC metabolite isomers. In addition, this is the first report confirming Δ^{10}-THCCOOH in authentic urine specimens. A trend was observed indicating that 9(*R*)-HHCCOOH may be the predominant stereoisomer in specimens containing one or more HHC parent stereoisomers and no THCCOOH. This was in contrast to HHCCOOH in combination with Δ^8- and/or Δ^9-THCCOOH, where 9(*S*)-HHCCOOH was most frequently encountered stereoisomer. Ultimately, results from this study demonstrated that a panel consisting of Δ^8- and Δ^9-THCCOOH was sufficient for identifying cannabinoid exposure in the majority of urine specimens during this timeframe. These data provide valuable insight for laboratories striving to maintain relevant cannabinoid panel offerings and for those seeking to improve understanding of metabolic profiles found in authentic urine specimens.

P52

Development and Validation of a Simple LC-MS/MS Drug Screen for 65 Compounds in Blood and Urine

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Introduction: Standardization of forensic toxicological testing has been a long-term collaborative effort within the toxicology community. Recent standards have been published regarding scope and sensitivity of testing for drug impaired driving investigations and drug facilitated crimes. Furthermore, requirements have also been published for mass spectral analysis and validation. However, the comprehensive screening of various classes of compounds (i.e., polarity, pKa) has shown difficulty with low resolution liquid chromatography tandem mass spectrometry (LC-MS/MS). Specifically, cannabinoid substances demonstrate complexity to extract in high recovery with other basic, acidic, and neutral compounds. Many studies that have combined these analytes utilize high resolution instrumentation (e.g., ion trap and time-of-flight). However, not all toxicological laboratories have access or funding for these technologies. Therefore, this study focused on developing and validating a low-resolution targeted LC-MS/MS drug screen in blood and urine that met all standard requirements published by the American National Standards Institute/Academy Standards Board (ANSI/ASB) for drug impaired driving investigations (#120), drug facilitated crimes (#121), mass spectral analysis (#098), and validation (#036).

Methods: Blood/urine (200 µL) was added to a plastic filter vial and fortified with 20 µL of control and 20 µL of internal standard. Protein precipitation was performed using 350 µL of cold acetonitrile (ACN). Vials were capped with filter pistons and pulse vortexed for 5 minutes at 2500 rpm[1]. The filtered piston was depressed for each sample, and 350 µL of ACN were transferred to a glass conical tube. The filtrate was evaporated under nitrogen for 15 minutes at 60°C. Samples were reconstituted in 30 µL of a 75:25 mixture of mobile phase A and B. Extracts were centrifuged at 4000 rpm for 5 minutes and transferred to an autosampler vial for LC-MS/MS analysis. Separation was achieved using a C18 column and gradient elution with aqueous 5 mM ammonium formate/0.1% formic acid and methanol. Validation parameters evaluated included limit of detection (LOD), ion suppression/enhancement, interferences, carryover, and autosampler stability. Each drug monitored a minimum of one ion transition for identification, in addition to, retention time, signal to noise, and ion ratios (as applicable).

Results: Multiple sample preparation methods were investigated to determine satisfactory recoveries of all targeted compounds including filter vials, supported-liquid extraction (SLE), protein precipitation, and in-tip filtration; however, filter vials exhibited the best recovery. For instance, zolpidem-COOH could not be recovered using SLE, and benzodiazepines had lower recoveries. The LOD was administratively set and evaluated at the cutoff concentrations defined in the ANSI/ASB standards. Low response recoveries were observed for certain classes of compounds such as barbiturates, cannabinoids, and low concentration analytes (e.g., buprenorphine); however, detection at the cutoff was reproducible and reliable. Some interferences and ion suppression were encountered; however, the method was determined to be fit for purpose as a presumptive toxicological screen.

Conclusion: This study demonstrates the utility of a low-resolution LC-MS/MS method for the comprehensive presumptive screening of 65 compounds in blood and urine. All compounds required by the ANSI/ASB standards for impaired driving and drug facilitated crime investigations were included. In addition, all essential validation parameters were evaluated, and each drug monitored a minimum of one ion transition for presumptive identification as well as other vital acceptance criteria as necessary by the ANSI/ASB standards.

[1] Farley, M., Tran, H., Towler, S., Gevorkyan, J., Pearring, S., and Rodda, L.N. (2021) A Single Method for 127 Recommended and Additional DUID Drugs in Blood and Urine by LC-MS/MS. Journal of Analytical Toxicology, 46, 658-669.

P53

Prevalence and Quantitative Analysis of 2F-2-oxo-PCE in Toxicology Specimens Collected in the United States

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Introduction: In recent years, the novel synthetic hallucinogen 2F-2-oxo-PCE has continued emerging across the United States, at times misidentified as its positional isomer fluorexetamine (3F-2oxo-PCE). Novel hallucinogens can cause similar dissociative effects to ketamine and phencyclidine (PCP) when taken recreationally. 2F-2-oxo-PCE and its isomers are not scheduled under the Controlled Substances Act but have potential for several adverse effects, including death. As of May 2024, 2F-2-oxo-PCE was identified in more than 35 toxicology specimens submitted to the Center for Forensic Science Research and Education (CFSRE), as well as 20 drug materials, including sample types purported as "fluorexetamine," "dope," and "K2." As novel hallucinogens become more frequently present in toxicology casework, it is pertinent to distinguish positional isomers and develop quantitative methods to accurately report reference blood concentrations.

Objectives: This study focused on developing and validating a new analytical method for the chromatographic resolution and quantitative analysis of fluorexetamine and 2F-2-oxo-PCE. Liquid chromatography tandem quadrupole mass spectrometry (LC-QQQ-MS) and quantitation via standard addition was used to determine the concentration of drug in authentic forensic casework that initially screened positive for fluorexetamine/2F-2-oxo-PCE by liquid chromatography quadrupole time-of-flight mass spectrometry (LC-QTOF-MS).

Methods: Forensic toxicology cases were routinely prepared for LC-QTOF-MS screening analysis using a basic liquid-liquid extraction (LLE) procedure. Instrumental analyses were completed with a SCIEX X500R LC-QTOF-MS system. Datafiles were processed against an internal library database of more than 1,100 analytes. During toxicological investigations, ten distinct case samples tested positive for fluorexetamine/2F-2-oxo-PCE and were confirmed by LC-QQQ-MS. Samples (0.5 mL) were prepared using a basic LLE. Instrumental analysis was performed using a Waters Xevo TQ-S Micro LC-QQQ-MS. Chromatographic separation of fluorexetamine and 2F-2-oxo-PCE was achieved on an Agilent InfinityLab Poroshell C-18 120 (2.7 µm, 3.0x150 mm) analytical column using gradient elution. Mobile phase compositions were 5mM ammonium formate in water, pH 3 and 0.1% formic acid in acetonitrile. Flow rate was 0.4 mL/min with an injection volume of 5 µL. Quantitation was assessed using standard addition and this method was validated using ASB standards 036 and 054. The calibration range was 0.2 to 50 ng/mL. Ketamine-D4 was used as the internal standard. Cases were up-spiked at 0.2, 2, and 20 ng/mL concentrations. Authentic toxicology specimens collected in for-cause scenarios and provided in collaboration with NMS Labs were analyzed with the developed method. Basic demographic information was available for seven of the ten cases received.

Results: Cases containing 2F-2-oxo-PCE were submitted from Michigan, Colorado, New York, Maine, and California between August 2023 and April 2024; and one case was provided from British Columbia, Canada. 50% of decedents were male and 50% were female, with most individuals between the ages of 20-28 years old. Based on the LC-QTOF-MS qualitative results, 2F-2-oxo-PCE was seen with traditional benzodiazepines (70%), opioids (40%), and stimulants (40%). Samples also contained other substances such as xylazine (40%) and other hallucinogens such as ketamine (30%). Based on the quantitative LC-QQQ-MS assay, all samples confirmed for 2F-2-oxo-PCE, and fluorexetamine was not detected. 2F-2-oxo-PCE blood concentrations ranged from 5.7 to >10,000 ng/mL. A subset of samples contained other novel psychoactive substances (NPS), including bromazolam (30%), flubromazepam (10%), and dimethylpentylone (20%).

Discussion: The validated standard addition method was successful in identifying, resolving, and quantifying fluorexetamine and 2F-2-oxo-PCE. From the casework samples analyzed thus far, the only encountered isomer is 2F-2-oxo-PCE. This synthetic hallucinogen was commonly detected alongside other drugs, many of which were CNS depressants. With the potential health concerns surrounding synthetic hallucinogen use, especially at high doses, forensic toxicologists should be aware of 2F-2-oxo-PCE. The positional isomer fluorexetamine can allow for misidentification of 2F-2-oxo PCE, and isomer differentiation is crucial for these new NPS in suspected hallucinogen cases.

P54

The Rise of Bromazolam in Postmortem Cases from Travis County, Texas and Surrounding Areas

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Introduction: Over the past decade, novel psychoactive substances (NPS) have continually emerged posing numerous problems for toxicologists. NPS benzodiazepines have recently grown in prevalence and pose significant public safety threats as they are often masked as traditional benzodiazepines like alprazolam. Bromazolam is the latest NPS benzodiazepine to emerge on the market following the scheduling of other NPS benzodiazepines. Like other NPS benzodiazepines, bromazolam is commonly found in combination with other drugs, particularly fentanyl. This combination has been coined "benzo-dope" and is thought to prolong the effects of fentanyl while also enhancing the risks of drug-induced toxicity. Overall, there is a paucity of bromazolam data detailing its pharmacology and toxicology.

Objective: Given the significant increase in fentanyl-related drug fatalities recently observed in Travis County, Texas (TX), and the surrounding areas, the international rise of bromazolam on the NPS drug market, and the increased occurrence of "benzo-dope" reports, we aimed to investigate the prevalence of bromazolam in our population. From 2021-2023, a retrospective review examining the bromazolam-positive deaths in Travis County and the surrounding areas was conducted to characterize the demographic and toxicological data.

Method: Postmortem cases submitted to the Travis County Medical Examiner for toxicology testing between January 1, 2021 and December 31, 2023, were queried to identify the total number bromazolam-positive deaths reported during this timeframe. Demographic (sex, race, age) and toxicological (bromazolam concentrations and polysubstance trends) information was gathered for all bromazolam-related fatalities, bromazolam-related drug toxicities, and non-contributory bromazolam cases, and evaluated annually and in totality.

Results: Bromazolam was identified in 112 deaths from 2021-2023, accounting for 1.57% of postmortem cases submitted for toxicology testing (n=7129). Significant increases in postmortem bromazolam-related drug toxicities were observed, with a 7.5-fold increase in deaths from 2021 (n=7) to 2023 (n=53). Of the 112 bromazolam-positive cases, 89% were ruled accidental (n=100). Bromazolam-related fatalities primarily occurred in males in their early-30's. Their concentrations ranged from 21-220 ng/mL, with mean (median) concentrations of 69.4±48.4 (53.5) ng/mL. There were two postmortem cases where bromazolam was attributed as the sole cause of death; postmortem blood concentrations were 39 and 23 ng/mL. Polydrug use was present in 98% of bromazolam-positive cases, with fentanyl (69%), amphetamines (43%), and other benzodiazepines (35%) representing the most concurrently identified drugs and/or drug classes. Bromazolam-related drug toxicities commonly involved fentanyl (82%), methamphetamine (41%), and cocaine and/or its metabolites (28%), whereas non-contributory bromazolam fatalities often involved methamphetamine (38%), alprazolam (33%), and cocaine and/or metabolites (33%). Initially, bromazolam was found with other benzodiazepines (>50%), however, their co-occurrence began to decline after Q3 2022. Roughly, 77% and 80% of alprazolam and other NPS benzodiazepine identifications occurred prior to Q4 2022, respectively. Starting in 2023, fentanyl was consistently identified with bromazolam (>80%).

Discussion/Conclusion: Significant increases in bromazolam-positive fatalities and bromazolam-related drug toxicities were observed in Travis County, TX from 2021-2023, emphasizing its public safety threat in this population. The increased prevalence of bromazolam with fentanyl was particularly alarming, especially in the context of the recent onset of the fentanyl crisis in Travis County. Significant concern for enhanced drug toxicity is warranted due to its polysubstance abuse. Furthermore, drug users may unknowingly consume these "benzo-dope" formulations or counterfeit pills (such as 'Xanax') containing bromazolam (and other substances) posing a danger to the community. With the continued rise of NPS and the threat they pose to the public, there is a continuing need for laboratories to adapt their testing scopes to include NPS that are often underreported to better understand these unregulated substances and their role in public health and safety.

P55

β-Glucuronidase Enzyme Kinetic Analysis for Applications in Urine Samples

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Introduction: Opiate and opioid metabolism primarily occurs in the liver, and these drugs are removed from the body by glucuronidation and sulfation to facilitate excretion in urine. Drug testing laboratories can quantify free opiates and opioids in urine by LC-MS/MS by hydrolysis with a β-glucuronidase and/or sulfatase. Herein, enzyme kinetics for morphine-3-glucuornide were measured and reported using two key enzyme characteristics: Michaelis-Menten constant (K_M) and turnover number (k_{cat}).

Objectives: Measure the K_M, V_{max} and k_{cat} of commercially relevant β-glucuronidase enzymes on morphine-3-glucuronide.

Methods: Deuterated and reference standards were purchased from Cerilliant. All reagents were purchased from MilliporeSigma or Fisher Scientific. Two commercially available β-glucuronidases are from two different manufacturers, and both are advertised as room temperature hydrolysis. The first commercially available β-glucuronidase is IMCSzyme® RT from IMCS. The second β-glucuronidase is from another manufacturer and referred to as Premixed Enzyme (PreEnz). Enzyme kinetic parameters such as Michaelis-Menten constant (K_M), velocity max (V_{max}) and turnover number (k_{cat}) were determined by LC-MS/MS.

Drug glucuronide standards for Michaelis-Menten kinetic experiments were dried using a CentriVap, reconstituted in water and concentrations confirmed by LC-MS/MS. Calibration curves included parent and glucuronide standards. All reactions were performed at room temperature and buffered at their recommended pH. Reactions were stopped by transferring sample into methanol (90%). Samples were further diluted and injected on a Thermo Scientific™ Vanquish™ UHPLC system coupled with a Thermo Scientific™ Endura™ Triple Quadrupole Mass Spectrometer. Analytes were separated using a Phenomenex Kinetex® 2.6 µm Biphenyl 100 Å, 50 x 4.6 mm column. Mobile phase A and B were 0.1% formic acid in water and 0.1% formic acid in acetonitrile, respectively.

Results: Lineweaver-Burk plots were used to calculate the K_M, V_{max} and k_{cat} on morphine-3-glucuronide for commercially available enzymes: IMCSzyme® RT and PreEnz. IMCSzyme RT is a mixture comprised of two different β-glucuronidases: R3 and EeGUS. Therefore, kinetic properties of both enzymes were tested separately. The K_M for R3, EeGUS and PreEnz were 0.88, 1.3 and 0.56 mM, respectively. The V_{max} for R3, EeGUS and PreEnz were 28, 14 and 35 µM/min, respectively. The k_{cat} of R3, EeGUS and PreEnz were 40, 8.3 and 3.3 sec^{-1}, respectively. The enzyme with the lowest K_M and highest V_{max} was PreEnz; however, R3 and EeGUS had higher catalytic efficiencies than PreEnz (about 12 and 2.5-fold higher, respectively) and require less enzyme to complete the reaction.

Discussion: Enzyme efficiencies are based on two features: affinity towards the target substrate (K_M) and the turnover rate (k_{cat}). Optimal characteristics for an efficient enzyme would include high affinity (low K_M) and high turnover rate (high k_{cat}) with a wide pH range toward relevant substrates. The experiments presented here were performed in buffered solutions, and R3, an enzyme in IMCSzyme® RT, had the highest turnover rate on morphine-3-glucuronide. This is important as prior studies have shown that for less efficient enzymes, such as PreEnz, more enzyme is required to complete hydrolysis of target drug glucuronides. When more enzyme is added to the reaction, the rate of LC-MS/MS column degradation, clogging and/or occlusion can increase, consequently increasing the cost associated with matrix analysis.

Biological matrices such as urine or blood would add another layer of complexity to enzyme kinetics and may include enzyme inhibition or inactivation due to endogenous compounds. Additionally, other compounds of interest include hydromorphone, oxymorphone, codeine, and acetaminophen as all of these drugs undergo glucuronidation and/or sulfation.

P56

Chemical and aerosol yield characterization of e-liquids: A 12-month study in parallel to participant use

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Introduction: E-cigarette (e-cig) use is now commonplace. Generations of e-cig devices and multiple e-liquid flavorings and nicotine concentrations have been marketed in the United States. Understanding the stability of e-liquid constituents is critical to assessing exposures from e-liquids that contain various nicotine concentrations.

Objectives: The following study characterized multiple chemical constituents in five e-liquid nicotine concentrations, 12-month storage stability, and aerosol produced by a mod device.

Methods: E-liquid products ranged from 0 mg/mL nicotine to 6.0 mg/mL and were prepared by the University of Maryland using a nicotine-free base e-liquid (i.e., Mardi Gras flavor, AVAIL vapor, LLC). E-liquids, stored in amber glass bottles, were kept refrigerated at 2-8 ºC and protected from light. A mod device (Aegis Mini, Shenzen Geekvape Technology Co., Ltd) was used with specified settings from Battelle for aerosol generation. Nicotine concentrations were blind during chemical characterization, stability testing, and for the clinical study. Chemical characterization of the five e-liquids determined concentrations of nicotine, propylene glycol (PG), vegetable glycerin (VG), N'-Nitrosonornicotine (NNN), and (4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone) (NNK) over 12 months. Other chemical properties studied were pH, free base nicotine, and PG:VG ratios. The aerosol studies measured yields of nicotine, PG and VG, carbonyls, and particle size distribution. Analytical methods spanned across LC-MS/MS, GC-FID, HPLC-PDA, and Laser Diffraction platforms. Aerosol collection was performed in accordance with CORESTA No. 81- Routine Analytical Machine for E-Cigarette Aerosol Generation and Collection.

Results: All e-liquids were stable under the prescribed conditions (stored at 2-8 ºC and protected from light) and the nicotine content remained within 5% of the Day 0 concentration over 12 months; therefore, these e-liquids are feasible for use in a long-term clinical study. PG:VG ratios also remained stable (i.e., 30:70 PG:VG). Aerosol yield provided differing characteristics for particle size, carbonyl content, and nicotine yield. The overall particle size average was 1.23 µm for each e-liquid at the Dv50 (distribution volume at 50%). Carbonyl yields ranged from below detection limits (acrolein) to 1.99 µg carbonyl per puff (formaldehyde). Nicotine aerosol yield ranged from 1.23 mg/g to 4.70 mg/g.

Discussion: This study shows that e-liquids ranging in nicotine content can be used in clinical studies over a period of at least a year without concern for overall product stability.

P57

Benzodiazepine Trends in Maryland Postmortem Cases: December 2023 – March 2024

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Introduction: Designer benzodiazepines (DBDZ), a subset of new psychoactive substances (NPS) modeled after therapeutic benzodiazepines (TBDZ), have emerged as substances of abuse. While TBDZ are regulated under Schedule IV of the Controlled Substances Act (CSA), five DBDZ were temporarily classified under Schedule I in 2023 due to their imminent hazard to public safety. Both TBDZ and DBDZ may be misused for their sedative effects to alleviate the acute withdrawal phase from stimulant use or to prolong the euphoric effects of opioids. Recently, bromazolam has been reported as the most prevalent DBDZ in various regions of the US. At Toxicology Laboratory in the Office of the Chief Medical Examiner (OCME), a new method was validated to quantitatively analyze nine TBDZ and seven DBDZ from biological specimens to better understand benzodiazepine-related deaths.

Objectives: This study aims to evaluate the efficacy of a newly developed gas chromatography tandem mass spectrometry (GC-MS/MS) method combined with a routine enzyme-linked immunosorbent assay (ELISA) for screening and quantitation of TBDZ and DBDZ. Additionally, we sought to investigate the prevalence of benzodiazepines in postmortem casework analyzed from December 2023 to March 2024, with a particular emphasis on DBDZ trends and usage during this period.

Methods: All cases were screened using the Immunalysis Benzodiazepine Direct ELISA Kit on a Dynex DSX system. Retrospectively, benzodiazepine-positive cases from the ELISA screen were analyzed by the GC-MS/MS confirmatory testing method. This GC-MS/MS method was validated according to ANSI/ASB Standard 036 and detects and quantifies 16 benzodiazepines, including seven DBDZ, with a detection limit ranging from 0.2-10 ng/mL in blood and liver matrices. Briefly, the method consists of a solid phase extraction, derivatization with MTBSTFA analysis by GC-MS/MS.

Results: Out of the 1,921 total cases submitted for toxicological analysis, 166 cases screened presumptively positive for benzodiazepines by ELISA, and 150 cases were confirmed by the new GC-MS/MS method, including 54 DBDZ cases (52 of which were bromazolam positive). Multi-drug use was found in 96% of the BDZ-positive cases. Fentanyl and its analogs were the most commonly co-occurring substances with BDZ, followed by cocaine. Decedents ranged in age from 8 to 89 years (median 53 years). The majority of the decedents were male (60.8%) and white (54.2%).

An increasing usage of bromazolam was observed, with 9, 13, 15, and 15 cases testing positive across December 2023, January, February, and March 2024, respectively. Bromazolam concentrations ranged from <5.0 ng/mL to 800 ng/mL, with a mean concentration of 110 ng/mL and a median of 51 ng/mL. Fentanyl and its analogs were present in almost all bromazolam users (51 out of 52 cases), cocaine was found in more than half of the bromazolam positive cases, and prescription CNS depressant drugs (TBDZ included) in about half of the cases. The demographics of bromazolam users differed from the overall population, with 58% African American and 38% white.

Discussion: The expansive benzodiazepine panel provides the Maryland OCME with a broader scope of testing and the capability to monitor DBDZ prevalence in the state. The dominant prevalence and increasing trend of bromazolam use in Maryland from the end of Q4 2023 to Q1 2024 align with national observations. Combining toxicology results from Driving Under the Influence of Drugs (DUID) cases and Drug-Facilitated Crime (DFC) cases in Maryland could offer a better perspective on benzodiazepine trends in the region. In the rapidly changing designer drug landscape, it will be necessary to periodically update the current panel by adding or removing DBDZ.

P58

Novel Psychoactive Substances Most Commonly Identified in Clinical Urine Specimens from November 2023 to April 2024

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Introduction: Novel psychoactive substances (NPS) are known as "legal highs," as they are designed to mimic the effects of recreational drugs while circumventing legality issues. These drugs began to increase in popularity in 2010, and, as of 2022, more than 1,000 different NPS have been identified. Testing for NPS is frequently performed in forensic laboratories for investigative and postmortem cases. Testing clinical specimens for the presence of NPS is helpful for understanding the full picture of patient compliance. Knowing which NPS are present in a patient specimen can also help to determine appropriate treatment as opioids, benzodiazepines, and stimulants would necessitate different treatment plans.

Objective: To identify which NPS are most commonly detected in clinical urine specimens submitted for testing to a reference laboratory.

Methods: A retrospective analysis of urine specimens submitted to Quest Diagnostics for NPS testing between November 2023 and April 2024 was used to determine positivity rates and trends for 85 NPS compounds in 6 NPS categories (designer fentanyl analogs, designer benzodiazepines, designer opioids, designer stimulants, synthetic cannabinoids, and other illicit compounds). Testing was performed using a semi-quantitative method that was previously validated according to CLIA and Quest Diagnostics requirements. A 3-point calibration curve was used, and all compounds had limits of detection between 0.5 ng/mL and 5.0 ng/mL.

Results: In total, approximately 6% of specimens were positive for at least 1 NPS. The majority (64%) of positive specimens were positive for only 1 of the 6 NPS categories, while 23% were positive for 2 categories, 9.1% were positive for 3 categories, 3.5% were positive for 4 categories, and 0.2% were positive for 5 categories. None of the specimens were positive for all 6 NPS categories.

The most commonly detected NPS was xylazine (other illicit compounds). However, xylazine positivity decreased over the study period, from 62.3% of positive specimens in November and December 2023 to 45.7% of positive specimens in March 2024. The most commonly detected NPS category was other illicit compounds (52.4% of positive specimens), and the second most commonly detected category was designer fentanyl analogs (38.3% of positive specimens).

The most commonly detected designer fentanyl analogs were fluorofentanyl (65.8% of designer fentanyl analog positives) and acetyl fentanyl (65.6%), and they were always detected with fentanyl and/or norfentanyl. The most commonly detected designer benzodiazepines were bromazolam (55.9% of designer benzodiazepine positives) and hydroxy-bromazolam (74.4%). However, in April 2024, positivity of flualprazolam and hydroxy-flualprazolam started increasing. The most commonly detected designer opioid metabolite was pyrrolidino hydroxy-nitazene (79% of designer opioid positives), and the most commonly detected designer opioid parent compound was pyrrolidino etonitazene (21.5%). The most commonly detected designer stimulant was dimethyl pentylone (85% of designer stimulant positives), which was frequently detected with its metabolite, pentylone. The most commonly detected synthetic cannabinoid was the butanoic acid metabolite for MDMB-4en-PINACA (98.8% of synthetic cannabinoid positives). The carboxylic acid metabolite of ADB-BUTINACA was the only other synthetic cannabinoid detected.

Discussion: In the specimens that were analyzed at Quest Diagnostics for NPS between November 2023 and April 2024, approximately 6% were positive for at least 1 NPS. Understanding the prevalence of NPS in the clinical population and the variety of compounds seen in clinical specimens can help physicians understand the importance of NPS testing to determine if a patient may be taking something else beyond drugs seen within the traditional scope of testing.

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Carfentanil and Other Fentanyl Analog Prevalence in Clinical Urine Specimens Analyzed Using a High-Throughput Novel Psychoactive Substances Testing Panel

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Introduction: Novel psychoactive substances (NPS) are known as "legal highs" and popularity of these compounds has increased exponentially over the last 15 years.

Among the designer fentanyl analog NPS category, acetyl fentanyl and fluorofentanyl are the most commonly seen analytes. Carfentanil, a designer fentanyl analog with potency 100-times that of fentanyl, has been a contributing factor in many overdose deaths across the country. However, carfentanil testing in samples from living individuals is not usually performed and therefore information on prevalence, observed concentrations, and clinical presentations in non-lethal scenarios is limited.

Objectives: The primary objective of this retrospective study was to understand prevalence and observed concentrations of carfentanil and other designer fentanyl analogs in recent clinical urine samples.

Methods: Results from Quest Diagnostics' NPS urine test from November 2023 through April 2024 were evaluated. Specimens were collected 1-4 days prior to analysis and NPS testing occurred when requested by the submitter. Specimens were analyzed after enzymatic hydrolysis, filtration, and dilution by a high-throughput LC-MS/MS method for 85 NPS compounds in 6 NPS categories (designer fentanyl analogs, designer benzodiazepines, designer opioids, designer stimulants, synthetic cannabinoids, and other illicit compounds). The method was validated according to CAP/CLIA guidelines, the American Academy of Forensic Sciences Standards Board, and Quest requirements. Limits of detection (LODs) ranged from 0.5 ng/mL to 5.0 ng/mL for all compounds and from 0.5 ng/mL to 2.5 ng/mL for the 18 fentanyl analogs specifically. Cutoffs for positivity were as follows for 3 of these analogs: acetyl fentanyl, 1 ng/mL; fluorofentanyl, 5 ng/mL; and carfentanil, 2 ng/mL. Quantitation was obtained using 3-point calibration curves. This linear curve was tested for accuracy up to 100 ng/mL.

Results: In total, 449 specimens were positive for acetyl fentanyl (26.0% of fentanyl positives) with concentrations ranging from 1.0 ng/mL to >100 ng/mL. For fluorofentanyl, 445 specimens were positive (25.7% of fentanyl positives) with concentrations ranging from 5.0 ng/mL to >100 ng/mL. Positivity for both drugs was observed in 225 specimens. Most specimens (65.8%) positive for fentanyl analogs also were positive for designer benzodiazepines, designer opioids, designer stimulants, and other illicit compounds. Carfentanil was observed in 23 clinical urine specimens (1.3% of fentanyl positives) with concentrations ranging from 2.3 ng/mL to >100 ng/mL. All fentanyl analog positive specimens were also positive for fentanyl/norfentanyl. These 23 carfentanil-positive specimens were also positive for xylazine, acetyl fentanyl, fluorofentanyl, bromazolam/hydroxybromazolam, and pyrrolidino hydroxynitazene. Clinical presentation at the time of these carfentanil-positive samples is unknown. All samples were from patients in substance abuse or prescription drug monitoring programs.

Discussion: In the clinical urine specimens submitted for NPS testing between November 2023 and April 2024, the fentanyl analogs most commonly seen were acetyl fentanyl and fluorofentanyl, consistent with previous studies. Surprisingly, 23 specimens were positive for carfentanil. Carfentanil is commonly tested in postmortem cases but rarely tested in clinical samples, therefore little is known about prevalence in this population. These results may indicate individuals have developed a tolerance to carfentanil, or other factors including pharmacogenetic differences, hydration status, time since last use, and frequency of use may contribute significantly to these observed urine concentrations. Further work is needed to characterize the metabolic profile of the active metabolite norcarfentanil in these urine samples and understand if prolonged urine excretion is similar to that observed with fentanyl and norfentanyl. Carfentanil detection in clinical urine samples provides information about patterns of use within the population of drug tested individuals.

P60

4-ANPP: The Caution Flag for Illicit Fentanyl

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Introduction: Fentanyl, a potent synthetic opioid, has seen a significant increase in non-medical use over the last decade contributing to a surge in overdose deaths and mortality rates. 4-Anilino-N-phenylethylpiperidine (4-ANPP), or despropionylfentanyl, is a minor metabolite of fentanyl. It also exists as a precursor in the Siegfried method of fentanyl synthesis, commonly employed for illicit production. Pharmaceutical grade fentanyl, manufactured via distinct methods, typically contains lower levels of 4-ANPP. Thus, the concentration of 4-ANPP may indicate if the fentanyl administered is illicit or pharmaceutical.

Objectives: This research project aimed to assess the capability of 4-ANPP as a marker for distinguishing pharmaceutical or illicit fentanyl use. Through the analysis of toxicology results from positive fentanyl and 4-ANPP cases in 2023, the concentrations of 4-ANPP in forensic submission samples were compared to those in clinical submission samples. The type of case, forensic or clinical, was delegated based on the submitting agency.

Methods: Quantitation of fentanyl and 4-ANPP was performed using High-Performance Liquid Chromatography/Tandem Mass Spectrometry (LC-MS/MS). The reporting limit (RL) for fentanyl was 0.2 ng/mL for blood and 0.5 ng/mL for serum. The RL for 4-ANPP was 0.2 ng/mL for all matrices. Analytical data was collected over one-year (January 2023–December 2023) and categorized by type of casework, either forensic or clinical, based on the submitting agency. Case history was not considered during data collection. Clinical case submitting agencies are primarily healthcare providers who have administered fentanyl in a hospital or trauma setting. It cannot be guaranteed that all fentanyl present was from hospital administration and not prior use, however, was highly likely based on the submitting agencies.

Results: A total of 32723 forensic blood cases were positive for fentanyl and 1015 clinical serum cases were positive for fentanyl and/or 4-ANPP. Forensic blood 4-ANPP results averaged 5.9 ± 40 ng/mL (range: 0-4100, median: 1.8 ng/mL), while clinical serum 4-ANPP results averaged 1.7 ± 11 ng/mL (range: 0-306, median: 0.15 ng/mL). Excluding cases where 4-ANPP was not detected, clinical serum cases averaged 3.7 ng/mL, while forensic blood cases averaged 6.5 ng/mL. The percent of non-detected 4-ANPP cases was 56% for clinical cases, compared to 9.2% for forensic cases.

Fentanyl concentrations were also included; forensic blood cases averaged 32 ±240 ng/mL (range: 0.2-30000, median: 14 ng/mL) and clinical serum cases averaged 13 ±29 ng/mL (range: 0.5-280, median: 3.5 ng/mL). Considering the blood-to-serum (b/s) ratio for fentanyl (0.8-1), forensic blood case statistics were converted, yielding an average of 40 ± 300 ng/mL (median: 17 ng/mL). The b/s for 4-ANPP is not currently known.

Discussion/Conclusion: Conclusions were formulated from this study on the assumption that 4-ANPP and fentanyl would behave similarly in serum. Although the b/s ratio for 4-ANPP is unknown, it has been considered that if 4-ANPP does not partition well in serum, then this would be a known limitation.

The rise in popularity of synthetic drug misuse has caused an increase in overdose related fatalities in the last decade. Fentanyl, used therapeutically for pain management and as an anesthetic due to its rapid onset of action, also has a high potential for misuse. The ability to differentiate illicit fentanyl from fentanyl administered by medical providers is crucial for medicolegal investigations. This study found the 4-ANPP concentrations were distinguishable between forensic and clinical cases. Forensic cases had a mean and medium that were 3 times and 12 times the clinical values, respectively. Additionally, the percentage of non-detected cases for clinical casework was 6 times higher than forensic. Despite some overlapping results, the overall difference in concentrations of 4-ANPP between forensic and clinical cases shows the possible capability of it being a significant indicator of the source of fentanyl administration.

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Breaking Bad: Methamphetamine's Role in Homicides

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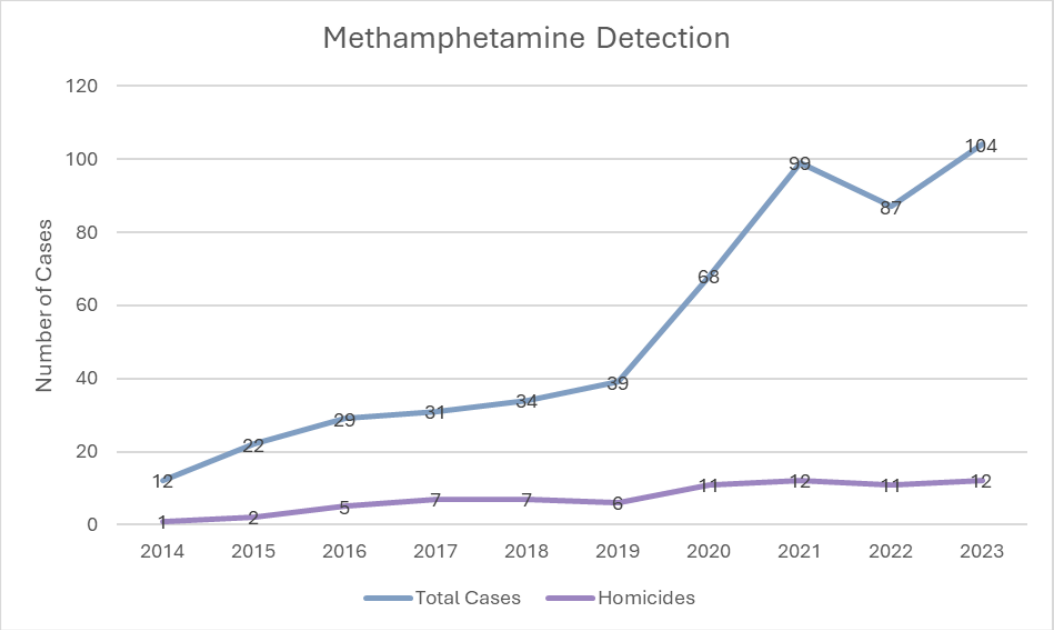
Introduction: Methamphetamine is a potent, highly addictive central nervous system stimulant utilized recreationally for its euphoric effects and its ability to increase energy and alertness. Methamphetamine is detrimental to users as it can cause dental problems, damage to the cardiovascular system, malnutrition, and memory loss. Additionally, methamphetamine use can lead to aggression and psychotic behavior which lends its reputation as the drug that contributes the most to violent crime in the United States. Since 2019, methamphetamine has regained popularity in Miami-Dade County as is evident in the significant increase in the number of post-mortem cases involving the drug, particularly in homicide victims.

Objectives: The objective of this presentation is to highlight the increase in the detection of methamphetamine at the Miami-Dade Medical Examiner Department (MDME). The presence of methamphetamine in homicide victims will be described as well as its presence in conjunction with other prevalent drugs, including cannabinoids, benzodiazepines, and ethanol.

Methods: Methamphetamine can be detected in multiple matrices by various methods at the MDME Toxicology Laboratory. These methods include solid phase or supported liquid extraction followed by detection utilizing a variety of instrumentation: gas-chromatography coupled with a nitrogen phosphate detector (GC-NPD), liquid chromatography triple quadrupole mass spectrometry (LC-MS/MS), or liquid chromatography coupled to an ion trap mass spectrometer (LC-IonTrap-MSn). Quantitation is performed using liquid chromatography triple quadrupole mass spectrometry (LC-MS/MS). Screening methods by LC-MS/MS and GC-NPD are performed routinely, while quantitation methods and confirmation with LC-IonTrap-MSn are performed as needed. All of these methods support analysis using multiple matrices such as blood, liver tissue, brain tissue, and urine. The laboratory's case management system was utilized to compile cases in which methamphetamine was identified during the period between 2014 and present. Cause of death (COD), manner of death (MOD), gender, age and race were included in the study.

Results: There were 543 cases with methamphetamine detected at the MDME from 2014 to present (Q1 2024). After 2019, there was sharp increase (166%) in the detection of methamphetamine in casework, with the most cases occurring in 2023 (104). Of all methamphetamine cases identified, 256 were accidental overdoses (47%), 77 were homicides (14%), and the remaining cases consisted of accidents unrelated to drugs, suicides, and natural causes. As noted in the graph below, homicides involving methamphetamine have doubled since 2019, following the increase of methamphetamine detection.



All the homicide victims are males between the age of 25 and 34 years-old (42%), and the majority of the homicides (76%) involved a firearm, with the cause of death resulting from gunshot wounds. The concentration of methamphetamine in various blood sources range from <0.010 mg/L to 15 mg/L.

Discussion: Following the COVID-19 pandemic, there has been a shift in illicit drug consumption, likely due to supply and demand changes. Now, some "traditional" substances that were previously few and far between have regained popularity, explaining the sharp increase in methamphetamine detection at the MDME. Despite not being the most prevalent stimulant in Miami, methamphetamine is beginning to impact homicide deaths. The stimulant effects of methamphetamine can cause erratic and aggressive behavior, which can lead to altercations with others, participation in dangerous activities, and trouble with law enforcement. Historically, cocaine has dominated the stimulant scene in Miami, followed by synthetic cathinones in more recent years. As of 2023, only 10% of deaths involving cocaine are homicides. Based on the increasing trend of methamphetamine presence in homicides over the last several years, it is hypothesized that the number will continue to increase in 2024.

P62

The Analysis of Drugs of Abuse (DoA) and Novel Psychoactive Substances (NPS) in Oral Fluids by LC-MS/MS

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Introduction: Testing for drugs in biological matrices is an important part of toxicology and workplace drug testing. The "gold standard" matrices that have been used for decades are typically blood and urine, however, the collection of these two matrices is invasive. The analysis of drugs of abuse in oral fluids is a solution that is gaining popularity due to its ease of collection compared to blood or urine collection. However, there are some issues with the buffer used in the collection devices when performing the oral fluid analysis. It can be difficult to remove all of the surfactants and preservatives present in the oral fluid collection device's buffer solution which can cause matrix effects and poor column lifetime. It can also be challenging to get full recovery of all of the analytes due to different techniques of emptying the sponge on the collection device. Often times solid phase extraction (SPE), or lengthy extraction techniques are utilized. Finding a workflow that uses a simple sample preparation paired with accurate and robust quantitation of the analytes is important for laboratories running these tests.

Objectives: The primary objective of this work is to demonstrate the analysis of drugs of abuse (DoA) and novel psychoactive substances (NPS) in oral fluids by LC-MS/MS using a salt-assisted liquid-liquid extraction (SALLE).

Methods: An LC-MS/MS method was developed using a Raptor Biphenyl 50 x 2.1 mm, 2.7 µm analytical column equipped with a Raptor Biphenyl EXP 5 x 2.1 mm, 2.7 µm guard column. Mobile phase A consisted of 0.1% formic acid in water and mobile phase B consisted of 0.1% formic acid in methanol. A total of 65 analytes were separated under gradient conditions, with a total cycle time of 10 minutes. This method utilized both positive and negative ESI modes. Samples were prepared in oral fluid and combined with Quantisal™ buffer. An aliquot from the buffer underwent sample preparation using a salt-assisted liquid-liquid extraction (SALLE). This technique used a saturated sodium chloride solution along with acetonitrile to extract the analytes from the matrix and buffer. The samples were dried down and reconstituted in 90:10 MPA: MPB.

Results: The biphenyl stationary phase offered superior selectivity and was able to resolve all 65 analytes, including all sets of isobaric analytes including, methamphetamine and phentermine, isotonitazene and protonitazene, and eutylone and pentylone among others. All sets of isobars had a resolution of 1.5 or greater, offering accurate quantitation of the analytes. Performance metrics such as recovery, linearity, matrix effects, and accuracy and precision were evaluated. All analytes included in this method passed both intra and inter-day accuracy and precision requirements, and no matrix interferences or effects were observed. Linearity was demonstrated using 1/x weighted linear regression and all analytes showed an r^2 of 0.99 or greater**.** An evaluation was completed, comparing dilute-and-shoot, which involved minimal clean-up, to the SALLE approach. Dilute-and-shoot failed to remove all of the buffer surfactants compared to SALLE. It was also difficult to achieve certain limits of detection using the dilute and shoot method for analytes such as buprenorphine. A recovery study on the collection device will also be discussed.

Discussion: A panel of 65 DoA and NPS were analyzed in oral fluid using a SALLE sample preparation technique and LC-MS/MS. This method demonstrates an accurate and robust solution for the analysis of these analytes. This method also offers a quick and efficient sample preparation procedure that cleans up with sample and removes buffer surfactants, without the need for SPE or other tedious extraction techniques, leading to faster processing of samples in high throughput laboratories.

P63

Meeting National Safety Council Recommendations: Accurate Rapid Tests and Laboratory Confirmation Procedures for Fentanyl and Prevalent Opioids in Oral Fluid – Part 2

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Introduction: Fentanyl and synthetic opioid use, abuse, overdose, and deaths are currently occurring at an alarming rate in the USA. Following a rapid test result, laboratory confirmation of an oral fluid specimen is required.

Objectives: This NIJ Grant Award had two objectives:

1. To address the unmet public health need and growing concerns around the opioid epidemic (including fentanyl), by producing a portable, visually read, inexpensive, accurate, reliable rapid oral fluid test device to provide users actionable information. (see Part 1)

2. To develop environmentally friendly confirmatory laboratory procedures for fentanyl, fentanyl analogs and synthetic opioids in oral fluid, using the same buffer as the developed rapid test device, so the same sample can be screened and confirmed.

Methods: Incorporating instrumentation widely available in forensic and clinical laboratories (LC-MS/MS), two separate environmentally friendly methods have been developed and validated according to professional laboratory standard ASB-036. The first confirmatory method included all the synthetic opioids on the rapid test strip, plus tramadol, buprenorphine, and methadone. The reason for the additions was the publication from ToxIC Fentanyl Study Group (CSFRE) that methadone, buprenorphine, and tramadol are seen frequently in NPS surveillance data. The second included fentanyl, several analogs and xylazine, because drug dealers may also mix adulterants (xylazine) and other drugs into liquid fentanyl solutions creating complex lethal drug mixtures to mimic the effects of similarly adulterated fentanyl powders and pills.

Both procedures are for specimen confirmation and incorporate low amounts of specimen (0.5mL = 0.125mL of neat oral fluid), organic solvents, buffers, and salts.

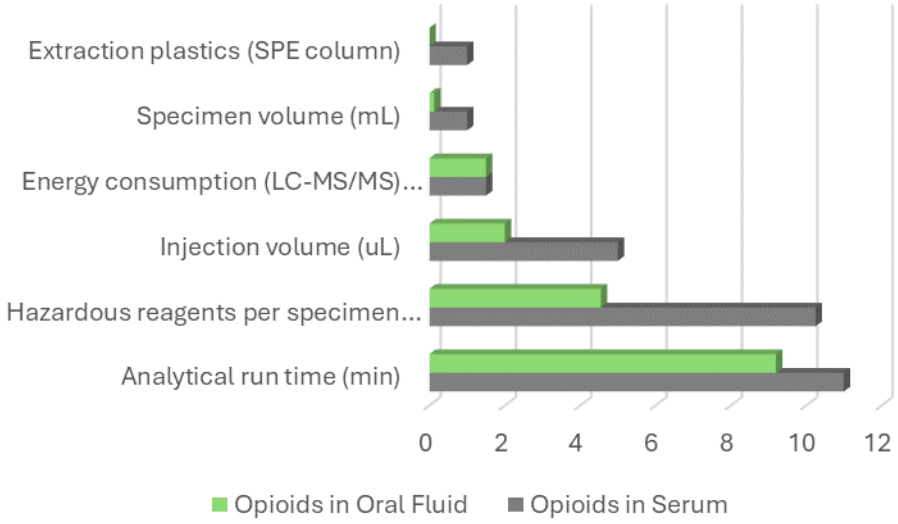
Environmentally friendly or 'green' laboratory method development was a specific goal for the confirmatory procedures. Non-green petroleum-based solvents (e.g. hexane) were replaced with bio-based bio-renewable solvents (e.g. 2-methyltetrahydrofuran) and incorporated bio-renewable versions of routine solvents (e.g. isopropanol and ethyl acetate) into the sample extraction process; lowered sample and reagent volumes and eliminated plastic solid-phase extraction columns.

Results: Our existing procedure for the extraction of opioids from serum was assessed and compared to the newly developed procedure for oral fluid based on 6 categories. The criteria of assessment evaluated were the choice and volume of reagents/solvents, energy consumption, sample volume, injection volume, plastics, and analytical run time. Where possible, bio-renewable solvents were used to replace routine organic solvents. Bio-renewables, also known as biomass-based products, are materials derived from biological sources that are renewable and can be used as substitutes for fossil fuels. Bio-renewable solvents are sourced from renewable, sustainable biobased materials, significantly lowering their environmental impact.

Discussion: The newly developed procedure was considerably "greener" than the currently existing method for opioids in serum. The breakdown of hazardous solvents used in the two procedures is shown:

Hazardous Reagents (total volume per specimen)	
Opioids in Serum	Opioids in Oral Fluid
Methanol (3.25mL)	Methanol (0.05mL)
Acetonitrile (2mL)	Bio-renewable isopropanol (0.5mL)
Acetic acid pH 4.0 (2mL)	Bio-renewable 2-Methyltetrahydrofuran (0.5mL)
Ethyl acetate (2.96mL)	Ethyl acetate (3.46mL)
Ammonium hydroxide (0.04mL)	Ammonium hydroxide (0.04mL)
Total: 10.25mL	Total: 4.55mL

This work was funded by NIJ Grant Award Number: 15PNIJ-23-GG-04233-RESS



P64

Meeting National Safety Council Recommendations: Accurate Rapid Tests and Laboratory Confirmation Procedures for Fentanyl and Prevalent Opioids in Oral Fluid – Part 1

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Introduction: Fentanyl and synthetic opioid use, abuse, overdose, and deaths are currently occurring at an alarming rate in the USA. The law enforcement community, the Food and Drug Administration (FDA) and the Centers for Disease Control (CDC) are all keenly aware of the urgency in addressing an unmet public health need to identify opioid overdose in individuals as rapidly as possible. Individual health outcomes will be significantly improved by allowing the correct treatments to be administered quickly. The device may also be useful in criminal justice and driving under the influence of drugs (DUID) cases.

Objectives: This NIJ Grant Award had two objectives:

1. To address the unmet public health need and growing concerns around the opioid epidemic (including fentanyl), by producing a portable, visually read, inexpensive, accurate, reliable rapid oral fluid test device to provide users actionable information.

2. To develop environmentally friendly confirmatory laboratory procedures for fentanyl, fentanyl analogs and synthetic opioids in oral fluid, using the same buffer as the developed rapid test device, so the same sample can be screened and confirmed (see Part 2).

Methods: In conjunction with our partners Orfila Diagnostics, competitive immunoassays for morphine with >80% cross reactivity to codeine, hydrocodone and hydromorphone; oxycodone (and oxymorphone); and 6-acetylmorphine in oral fluid have been developed for testing at cut-off concentrations required by SAMHSA for workplace testing and as recommended by the National Safety Council's Alcohol, Drugs and Impairment Division in driving under the influence of drug (DUID) cases and motor vehicle fatalities (2021 Update). The tests are incorporated into a lateral flow rapid test format. The specimen collected is approximately 1mL of neat oral fluid which is then diluted 1:4 with buffer in order to a) recover drug from the pad, and b) ensure drug stability in transport and storage. Drug recovery, transport and storage stability were determined. Assay specificity and interference testing with commonly ingested substances were determined.

Results: Significant progress towards a rapid test for synthetic opioids in oral fluid has been made. The competitive assays for the opioids at 30ng/mL cut-off concentration have been characterized and optimized; the assay for 6-acetylmorphine at a cut-off concentration of 4 ng/mL has also been developed. Sourcing, characterizing, and optimizing fentanyl antibodies for use in a lateral flow immunoassay is on-going. The sensitivity required for identifying fentanyl in oral fluid (currently suggested at 1ng/mL) is challenging in a lateral flow format. Recovery from the pad for all drugs was >90%; and all were stable in transport and in storage.

Discussion: Performance characterization of the oral fluid rapid test is visualized in the next few months. Clinical specimens from opioid users (including fentanyl) will be analyzed according to environmentally friendly laboratory confirmation methods (see Part 2).

P65

Suspected occupational exposure to fentanyl & derivatives: responses from a pilot survey of emergency physicians

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Introduction: The opioid overdose rate has risen in recent years primarily driven by synthetic opioids such as fentanyl and its derivatives. Suspected occupational exposures to opioids have been reported by first responders.

Objective: This pilot study aims to evaluate the possible symptom presentation of first responders following potential occupational exposure to fentanyl and its derivatives.

Methods: An anonymous online survey was sent to 14,072 emergency physicians of which 136 valid (i.e., completed by invited participants that were not traceable to a location outside of the US or a virtual private network) responses were included in the analysis. The survey solicited responses regarding signs and symptoms consistent with opioid toxicity, analytical confirmation of fentanyl or derivative presence in blood or urine, whether naloxone was administered, and improvement of the condition.

Results: The primary route of purported exposure was dermal with a quarter of all cases presenting symptoms consistent with the opioid toxidrome. Blood or urine were tested in less than a third of cases for the presence of fentanyl and its derivatives. Naloxone was administered in 8.6% of cases to reverse symptoms. The primary symptoms reported in the emergency department were respiratory depression (38.5%), somnolence (30.8%), and unconsciousness (23.2%).

Discussion: This pilot survey indicates that suspected occupational fentanyl and analogue exposures may be problematic. The results of this survey, though preliminary, should inform toxicologists, emergency physicians, and staff to be vigilant with suspected occupational fentanyl exposures, triage according to symptom presentation, and consider opioid toxicity as a cause. The lack of testing and confirmation for fentanyl/synthetic opioid exposure needs to be addressed to ensure accuracy of presence of opioids as patients are being triaged.

P66

Xylazine and 4-Hydroxy Xylazine Addition to Definitive Drug Testing Panel for Human Urine

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Introduction: Xylazine is a non-opioid CNS depressant authorized for veterinary use in the USA as an anesthetic sedative and muscle-relaxant analgesic. Illicit xylazine use has grown and is increasingly reported as an adulterant in drug seizures. This presentation focuses on addition of xylazine and its major metabolite 4-hydroxy xylazine to an existing definitive drug screening panel.

Objectives: To expand a definitive drug screening panel in urine by including xylazine and 4-hydroxy xylazine identification and quantitation. Previously, we reported a novel high performance liquid chromatography-mass spectrometry (UPLC-MS/MS) method for the detection/ quantification of 102 drugs/metabolites in urine. We now report method expansion for xylazine-use monitoring, with validation according to ANSI/ASB Standard 036.

Methods: Xylazine testing was added to an original UPLC-MS/MS screen for 102 analytes (Current Protocols 2022 3:e644). The method employs a novel matrix normalization technique (Threshold Accurate Calibration or TAC) involving testing of samples with (spiked) and without (neat) the addition of a known amount of reference analytes. Chromatographic separation was completed in 3 min using a Waters ACQUITY™ UPLC™ I-Class equipped with an ACQUITY UPLC BEH Phenyl column (1.7 µm, 2.1 x 50 mm) interfaced with a Waters Xevo® TQD mass spectrometer. Multiple reaction monitoring for xylazine (221.1>89.9 and 221.1>164.0) and 4-hydroxy xylazine (236.9>89.9 and 236.9>136.9) was performed in positive electrospray (ESI+) mode. MS data for the 104 analytes was analyzed using MassLynx® software and exported to a laboratory-developed Excel template for calibration, QC, and case analysis. Matrix normalized TAC ratio = neat peak-area response/(spike peak-area response – neat peak-area response). Methapyrilene was used as recovery standard for injection volume accuracy; analysis acceptance criteria were employed for ion ratio, chromatographic retention time, injection recovery, and calibration performance. Xylazine and 4-hydroxy xylazine calibrations were validated with use of either linear or quadratic equations over a calibration range of 38-400 ng/mL.

Results: Precision of xylazine and 4-hydroxy xylazine analysis showed an average percent coefficient of variation ranging from 6.8-10.1% with a bias ranging from -11.9 to -2.2%. Multi-run calibration R^2 ranges for xylazine (0.994-0.999) and 4-hydroxy xylazine (0.998-1.000) were determined with calibrators using a within 20% of target acceptance criteria. Carryover after ULOQ calibrator averaged 2.7% for xylazine and 2.9% for 4-hydroxy xylazine. Matrix effect in nine urine matrices supplemented with 50 and 400 ng/mL revealed ion enhancement for both xylazine (mean 7-16%) and 4-hydroxy xylazine (13-18%) with variability from 10-13% CV. Analytical specificity was validated with ten analyte-negative urine analyses and 124 commonly tested analytes. LOD was validated for analytes by supplementation at the LOD concentration (10 ng/mL) in nine analyte-negative urine pools. Process stability was assessed by repeat analysis of three control pools 18 hours after the initial analysis time (0 hours) with recovery for xylazine (94-107%) and 4-hydroxy-xylaxine (98-106%) determined. Dilution integrity was determined by analysis of urine control pools containing 900 ng/mL of xylazine and 4-hydroxy xylazine, using x2, x5, and x10 dilutions with recovery criteria of 20% target concentration. Initial casework findings in eight positive cases revealed xylazine and 4-hydroxy xylazine concentration ranges of 58-1090 ng/mL and <38-133 ng/mL, respectively. Testing with and without enzymatic hydrolysis revealed 45-63% glucuronidation of 4-hydroxy xylazine without evidence of xylazine conjugation. Xylazine positive cases were consistently associated with fentanyl use and other drug findings included gabapentin, cotinine, benzoylecgonine, morphine, and 6-acetylmorphine.

Discussion: We report the update of a definitive toxicology screen in urine to include xylazine and 4-hydroxy xylazine. Most validation studies were conveniently conducted during routine analytical runs for the other panel analytes, prior to implementation of xylazine testing in casework samples. The method was validated according to current forensic standards and initial application in routine casework confirms xylazine misuse within our region and also demonstrates the relative detection sensitivity of xylazine and major metabolite testing.

P67

Analysis of 15 cannabinoids in postmortem fluids and tissues using UPLC/MS/MS

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Introduction: Between FY2019 to FY2023, delta-9-tetrahydrocannabinol (delta-9-THC) has consistently been in the top 6 drugs detected by the Federal Aviation Administration's (FAA) Forensic Sciences Laboratory. However, in FY2022, the laboratory detected its' first delta-8-THC positive. In FY2023, 8.3 % of cases tested by the FAA Forensic Sciences Laboratory were positive for either delta-8-THC or delta-9-THC (or their metabolites). Of these positive cases, 28.6% contained delta-8-THC alone or in combination with delta-9-THC. Since approximately 60% of cases received by the FAA Forensic Sciences Laboratory include blood, it was crucial to develop and validate a method not only capable of separating numerous cannabinoids (including multiple isomers), but also suitable for the quantitation of such cannabinoids in alternative postmortem matrices.

Objective: To develop and validate a solid phase extraction and ultra-performance liquid chromatography tandem mass spectrometry (UPLC/MS/MS) quantitation method for 15 unique cannabinoids in postmortem fluids and tissues.

Methods: A panel of 15 cannabinoids was validated following the FAA Forensic Sciences Laboratory in-house validation procedure that consists of the following tests: linearity, bias and precision, stability (refrigerator, freeze/thaw, and post-extraction), alternative matrix controls, dilution controls, matrix effects, recovery, process efficiency, cross contribution, and drug interference. The extraction method utilized UCT Clean Screen THC solid-phase extraction cartridges to isolate cannabinoids from 0.5 mL of biological fluid or 0.5 g of tissue (1.5 g tissue homogenate). Analysis was performed on a Waters Xevo TQ-S UPLC system with an Acquity UPLC BEH C18 column (2.1 x 100mm, 1.7 µm) at 60°C. Mobile Phase A (MPA) was water with 0.1% formic acid and Mobile Phase B (MPB) was methanol with 0.1% formic acid. Flow rate was 0.5 mL/min with a sample injection volume of 1 µL. The LC gradient is displayed in the table below.

Time	MPA %	MPB %	Curve*
0.0	50	50	Initial
0.5	50	50	6
3.0	28	72	6
6.0	25	75	6
7.5	22.5	77.5	6
9.0	5	95	6
10.0	5	95	6
11.0	50	50	1

*Gradient curve is the rate of change in solvent composition over time, with lower numbers denoting a more rapid initial change in mobile phase composition when compared to a standard linear change (curve=6). Listed settings are for Waters LC software.

Results: The method was validated for the quantitation of: delta-8-THC, delta-9-THC, (6aR,9R)-delta-10-THC, (6aR,9S)-delta-10-THC, 11-hydroxy-delta-9-THC, delta-8-carboxy-tetrahydrocannabinol, delta-9-carboxy-tetrahydrocannabinol, delta-9-THC acetate (THCO), delta-9-tetrahydrocannabiphorol (THCP), delta-9-tetrahydrocannabivarin (THCV), cannabigerol (CBG), cannabinol (CBN), cannabidiol (CBD), 7-hydroxy-CBD, and 7-carboxy-CBD. All analytes showed a limit of quantitation (LOQ) in blood of either 0.39 or 0.78 ng/mL, except for 7-OH-CBD which had a LOQ of 1.56 ng/mL. There were limitations for the analysis and quantification of certain analytes. Analytes that did not have an isotopically matched internal standard, including (6aR,9R)-delta-10-THC, (6aR,9S)-delta-10-THC, THCO, and THCP, were unable to be quantitated in tissue specimens due to inequivalent recovery between the analyte and the internal standard used. However, positives tissue specimens can be reported qualitatively. Additionally, this method was unable to separate 11-hydroxy-delta-8-THC and 11-hydroxy-delta-9-THC so 11-hydroxy-delta-9-THC was used for calibrators and controls. Studies were performed

to assess the effects of 11-hydroxy-delta-8-THC when quantitated against an 11-hydroxy-delta-9-THC curve, and it was determined that accurate quantitative results were not reliable when the 11-hydroxy-delta-8-THC concentration exceeded that of the 11-hydroxy-delta-9-THC concentration. Therefore, since the contribution of 11-hydroxy-delta-8-THC to a mixed 11-hydroxy-THC peak cannot be determined in casework, delta-9-THC and/or delta-9-THC-COOH positive samples containing delta-8-THC and/or delta-8-THC-COOH will be reported qualitatively. Further, samples positive for only the delta-8 will have its hydroxy reported as qualitative. While we were not able to obtain a 11-hydroxy-delta-10-THC standard to perform similar studies with, this analyte will be treated the same as the 11-hydroxy-delta-8-THC.

Conclusion: This method was able to identify 15 cannabinoids in postmortem fluids and tissues. All analytes were found to be suitable for the quantitation of cannabinoids in postmortem fluids. However, analytes without isotopically matched internal standards were unsuitable for the quantitation of cannabinoids in postmortem tissues. Future research will include performing a postmortem distribution study for these cannabinoids by analyzing available fluid and tissue specimens from cases that previously tested positive for delta-9-THC only, delta-8 THC only, and both delta-8 and delta-9-THC.

P68

Profiling Heavy Metals in Kratom Powder Purchased in Southeastern Pennsylvania and Southern New Jersey

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Introduction: Kratom is a tropical tree native to Southeast Asia whose products are known to induce both stimulant and opioid-like effects arising from the presence of mitragynine and 7-OH-mitragynine. Despite the FDA's assertion that kratom derived products are not appropriate as dietary supplements, they have become increasingly popular due in part to support from advocacy groups such as the American Kratom Association. Kratom products are available in a wide variety of different forms including powders, capsules, teas, tonics, edibles and seltzers. Cardiovascular, gastrointestinal, neurological and psychiatric effects attributed to the active ingredients in Kratom have been reported. As a plant, the potential exists for phytoaccumulation of heavy metals derived from the soil in which the kratom is grown. Furthermore, the metals profile and concentrations extracted from the soil are highly susceptible to the environmental practices of the geographic region from which the plant originates. Several studies have been conducted on the potential for heavy metal toxicity in locally sourced kratom based products with published reports exhibiting varying results.

Objectives: The objective of this study is to add to the growing body of literature surrounding the potential for metals contamination of kratom based products. This will be done by documenting the heavy metals profile of locally sourced kratom powders of different strains (i.e. red, green white, etc.) purchased in southeastern Pennsylvania and southern New Jersey. The results will be compared with what has been reported in localized studies from Chicago, Ill and Richmond, VA.

Methods: Kratom powder was purchased locally from "head shops", "smoke shops", and gas stations in southeastern Pennsylvania and southern New Jersey. A total of seventeen samples were collected representing the three major strains (red, white and green kratom), four different countries of origin (Thailand, Vietnam, Malaysia, and Indonesia), and five different manufacturers. Powders were chosen as they are the consumer product that is least processed. Consequently, there is a lower potential for external heavy metal contamination. Samples were stored in the dark and refrigerated in their original packaging until processed for analysis.

Samples were prepared using closed vessel, microwave assisted digestion with each batch including a digestion blank and QC standard followed by ICP-MS analysis. Three replicate digests of each sample were analyzed. An initial qualitative, full periodic table screen was performed to identify elements of toxicological interest present at elevated concentrations and confirmed by a quantitative analysis of the presumptive results.

Results: A digestion protocol similar to that used for the analysis of trace metals in cannabidiol (CBD) oils previous published by this research group was found suitable for the kratom powders. The preliminary screen suggested that Mn, Ni, and Pb might be present at elevated concentrations in several of the samples tested and the presumptive results verified via a full quantitative analysis with an appropriate quality control schema.

Discussion: The potential exists for heavy metal contamination in kratom powder which would in turn call into question the heavy metals profile of other kratom containing consumer products such as tonics and edibles. Ongoing work is required to determine if there is a correlation between heavy metal levels and kratom species, geographic source (if known), and mitragynine concentration. The American Kratom Association has put into place a Good Manufacturing Practice (GMP) Standards Program that holds member manufacturers to adhere to a rigorous standards program entitling them to use the "AKA GMP Qualified" seal on its label and labeling. The effectiveness of that program relative to non-member manufacturers also merits further study. In this investigation, none of the samples analyzed had been certified by the American Kratom Association.

P69

Flash Enzymatic Hydrolysis Protocols for Different Drug Class Using B-One®

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Introduction: Glucuronidation is a crucial stage within the elimination process of many drugs from the body. This metabolic process involves the conjugation of drugs with glucuronic acid, forming more polar compounds that can be more easily eliminated, mainly through urine. Consequently, these conjugated drugs can complicate drug analysis by mass spectrometry for multiple reasons and to help resolve this issue, many laboratories utilize beta-glucuronidase enzymes. This approach allows for more accurate detection and quantification of these drugs and metabolites while streamlining the analytical workflow by converting glucuronide-conjugated metabolites back to their original, parent form. This eliminates the need for analyzing both free and conjugated forms, reduces sample run time, enhances detection sensitivity, and overall improves efficiency. However, it's important to take into consideration that utilizing a universal approach when hydrolyzing different drug classes such as benzodiazepines, cannabinoids, and antidepressants, may not be the most effective option. One of the reasons for this is that each analyte has a unique interaction with beta-glucuronidase enzyme, hence it is necessary to perform a customized hydrolysis method considering: the drug classes involved, specific recovery needed, or the analysis of targeted compounds.

Objective: Our main aim is to emphasize that an optimized approach allows hydrolyzing different drug classes rapidly, at room temperature, with adequate recoveries.

This study illustrates the relevance of employing best practices with Finden enzymes while evaluating the hydrolysis performance using B-One® (Finden® by Kura Biotech®) recombinant beta-glucuronidase for the various drug classes tested in forensic and clinical toxicology laboratories.

Materials and Methods: Common drug class analytes were quantitatively determined in different panels for hydrolysis efficiency using B-One®, standards prepared at a ULOQ of 2500 ng/mL, incubation at room temperature, different incubation times (1, 5, 15 minutes), and the following conditions:

Drug Class	**Free Analyte**	**Conjugated**	Urine: B-One® ratio
Benzodiazepines	Oxazepam	Oxazepam glucuronide	5:2
	Temazepam	Temazepam glucuronide	
	Lorazepam	Lorazepam glucuronide	
OUD Medication	Buprenorphine	Buprenorphine-3ß-D-glucuronide	1:1
	Norbuprenorphine	Norbuprenorphine glucuronide	
	Naloxone	Naloxone-3ß-D-glucuronide	
Tricyclic Antidepressants (TCA)	Amitriptyline	Amitriptyline N-β-D-glucuronide	1:1
Cannabinoids	11-nor-9-Carboxy-Δ9-THC	11-nor-9-Carboxy-Δ9-THC glucuronide	5:3

Additionally, the OUD Medication panel follows a clean-up protocol using XTR™ tips 5 mg HLB (DPX Technologies) and then diluted with DI water for analysis by LC-MS/MS.

Results: The proposed protocols for the different drug classes will allow significant recoveries of the analytes of interest while employing a room-temperature incubation time of 15 or fewer minutes to achieve a streamlined sample preparation protocol.

Discussion and Conclusion: The hydrolysis experiments conducted on various analytes of interest for different drug classes demonstrate the need for customized hydrolysis parameters for B-One® including different hydrolysis times and urine-to-enzyme ratios.

P70

Direct-to-Definitive Drug Testing in Oral Fluid: Development of a Convenient Passive-drool Collection Technique and Evaluation of Analyte Stability

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Introduction: Oral fluid (OF) drug testing is applicable in forensic casework, with advantages of a noninvasive and inoffensive observed-collection with low risk for adulteration. Recognizing the value of direct-to-definitive drug testing, we developed and validated a high performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS) method for screening and confirmation of 99 drugs/metabolites (analytes) in buffered neat OF. The stability of drugs in OF testing is known to be a major pre-analytical challenge and validation of analyte stability is therefore essential in forensically defensible OF testing.

Objectives: To develop a convenient passive-drool collection of OF and to evaluate the pre-analytical stability of 99 analytes in a definitive drug testing method. OF analyte stability was evaluated with acidified and neutral buffering of neat OF, as well as with a commercial absorbent pad method of specimen collection and stabilization.

Methods: Neat OF (1 mL) was obtained by passive-drool collection using a SalivaBio Saliva Collection Aid fitted into a septum-capped glass vial. Routine collection of neat OF employed buffering with 50 mM citrate buffer (pH 7.0). Comparative stability studies with the routine collection method, using pre-analytical specimen storage at 2-4°C for up to 28 days, were performed using both neat OF acidified to pH 3.8 with formic acid and a commercial Saliva Sampler (StatSure) diluent. The definitive testing method employs a novel matrix normalization technique (Threshold Accurate Calibration or TAC) that involves testing of the OF samples with (spiked) and without (neat) the addition of a known amount of reference analytes. Chromatographic separation was completed in 3 min using a Waters ACQUITY™ UPLC™ I-Class equipped with an ACQUITY UPLC BEH Phenyl column (1.7 µm, 2.1 x 50 mm) interfaced with a Waters Xevo® TQD mass spectrometer. Multiple reaction monitoring (MRM) was performed in positive electrospray (ESI+) mode. MS data from dual transition MRM ion acquisitions was analyzed using MassLynx® software with data export to a laboratory-developed Excel template for calibration, QC and case analysis, based on matrix normalized TAC ratio = neat peak-area response/(spike peak-area response – neat peak-area response). Methapyrilene was used as a recovery standard for injection volume accuracy; analysis acceptance criteria were employed for ion ratio, chromatographic retention time, injection recovery, and calibration performance.

Results: The septum-fitted OF collection aid provided a simple and inoffensive method of collecting a defined volume of OF, with ability to add known preservatives. Analytical recovery, with mean (%CV) precision in over 10 analytical runs of 98.1% (11.1%), 100.1% (9.3%) and 106.2% (6.2%), was determined for neat OF (pH 7.0 buffered) control pools containing analytes at 40%, 125% and 500% of LLOQ concentration, respectively. Analyte stability criteria (within 20% of freshly prepared analyte concentration) in unbuffered OF at 2-4°C was not met for multiple analytes during a two week storage period study. Degraded analytes included cocaine (COC) and methylphenidate (MP), with concomitant increases in concentrations of their respective metabolites, benzoylecgonine and ritalinic acid. Both pH 7.0 buffering and the commercial preservation maintained stability of all analytes for at least 7 days at 2-4°C, with stability of up to 28 days for most analytes. Acidification of neat OF allowed stabilization of COC, MP and their metabolites over a 28 day period but resulted in significant degradation within 24 hours for buprenorphine, norbuprenorphine, clonazepam, nordiazepam, 4-ANPP and olanzapine.

Discussion: We report a convenient passive-drool collection system for neat oral fluid. Neutral buffering of OF and storage at 2-4°C demonstrated pre-analytical stability of all analytes up to 7 day, with stability up to 28 days for most analytes. A commercial preservative provided similar stability but acidification of OF lead to significant degradation of multiple analytes within the first 24 hours.

P71

DUI Snapshot: Uncovering Drug Prevalence Trends in Washington, D.C.

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Introduction: Driving under the influence (DUI) continues to pose a significant challenge to traffic safety in the Washington, D.C. metropolitan area. Despite concerted efforts in enforcement and public awareness campaigns, the intersection of substance use and impaired driving persists as a complex issue. This study endeavors to shed light on the patterns of drug-related incidents associated with DUI offenses in Washington, D.C.

Objectives: The primary objective of this study was to assess the prevalence and patterns of drug use in DUI cases in Washington, D.C. spanning the years 2016 to 2023.

Methods: DUI cases from 2016 to 2023 were sourced from various law enforcement agencies, including the Metropolitan Police Department, United States Secret Service, United States Park Police, United States Capitol Police, and Central Intelligence Agency. Samples for analysis, which could include blood, urine, and/or serum, were received and screened at the Office of the Chief Medical Examiner's Forensic Toxicology laboratory in Washington, D.C. Blood and serum specimens were screened by enzyme-linked immunosorbent assay (ELISA), and analyzed for volatiles (including ethanol, methanol, isopropanol, and acetone). Urine specimens were consistently analyzed for volatiles and synthetic cannabinoids (LC/MS/MS) during the monitoring period. Urine specimens analyzed before November 2019 were screened by ELISA. Subsequently, the ELISA screen was replaced by homogenous enzyme immunoassay (HEIA). Headspace-Gas Chromatography/ Flame Ionization Detector, and liquid-chromatography/ mass spectrometry/ mass spectrometry were utilized for volatiles and drug confirmatory analysis, respectively. The final results of 3,417 DUI cases (averaging 427 cases annually) were compiled and statistically analyzed using Microsoft Excel.

Results: Over the 8 years analyzed, the most prevalent drugs were consistently: ethanol, delta-9-tetrahydrocannabinol (THC) / 11-nor-9-carboxy-delta-9-tetrahydrocannabinol (THC-COOH), and phencyclidine (PCP) followed by benzoylecgonine/cocaine/cocaethylene and fentanyl/norfentanyl. The ethanol positivity rate peaked in 2022 and 2023, reaching 94% each year, despite receiving fewer cases than in preceding years. During the same time period, PCP positivity peaked in 2016 at 40% and declined in 2022, finally reaching its lowest at 13% in 2023. The THC/THC-COOH positivity rate exhibited an upward trend throughout the monitoring period, peaking in 2021 and 2022 at 61% each year. Ethanol was the most frequently encountered drug followed by PCP, and THC/THC-COOH in 2016. For the remainder of the monitoring period (2017–2023), the prevalence remained constant, with ethanol ranking highest, trailed by THC/THC-COOH, and PCP, with average positivity rates of 85%, 16%/57%, and 28%, respectively. Of the 3,417 cases, only 206, roughly 25.7 cases per year, had no drugs detected.

Discussion: Washington, D.C. presents a unique landscape where PCP remains a prominent substance used, distinguishing it from many other geographical areas. However, the decline in PCP detections in DUI cases in 2022 and 2023 contrasts with previous trends. The decriminalization of cannabis in Washington, D.C. (2015) and neighboring areas like Virginia (2021) and Maryland (2023), resulted in the highest THC/THC-COOH detection rates ever seen in the District. DUI toxicology findings are crucial tools in assisting legal and law enforcement protocols. They offer vital insights into the prevalence and patterns of intoxicating substances, thereby facilitating the development of more robust approaches for enforcing laws about driving under the influence.

P72

The Rise of Nitazenes in Alabama

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Introduction: The prevalence of synthetic opioids, such as nitazenes, in the United States has increased in recent years. Synthetic opioids are narcotic analgesics that are commonly found in powder or tablet form and administered through oral, nasal, and intravenous methods, resulting in possible respiratory depression, convulsions, and fatal outcomes.

Objectives: To validate N-pyrrolidino protonitazene, N-pyrrolidino metonitazene, and N-desethyl isotonitazene targets qualitatively by Liquid Chromatography Tandem Mass Spectrometry (LC/MS/MS) and to review the Alabama Department of Forensic Sciences (ADFS) toxicology data regarding nitazene involvement in all case types from January 1, 2021, to June 1, 2024.

Methods: Validation studies were conducted per ANSI/ASB Standard 036 for the qualitative addition of three new targets: N-pyrrolidino protonitazene, N-pyrrolidino metonitazene, and N-desethyl isotonitazene using an Agilent 6460 LC/MS/MS. Cross-reactivity studies were performed with the Randox Evidence Analyzer Plus.

Demographics and the most common drugs used in combination with nitazenes were determined. Three case studies were highlighted, explaining case-specific information. The study monitored various nitazenes, including isotonitazene, metonitazene, etonitazepyne (n-pyrrolidino etonitazene), butonitazene, protonitazene, etodesnitazene, etonitazene, N-pyrrolidino protonitazene, N-pyrrolidino metonitazene, and N-desethyl isotonitazene.

Results: The newly validated nitazenes were qualitative by LC/MS/MS after completing validation studies. The LOD for the new targets was 0.5 ng/mL. Current nitazene targets did not exhibit cross-reactivity on the Randox Evidence Analyzer Plus with the Ultra biochip.

In 2021, 2022, and 2023, two, eight, and 14 nitazenes were reported in casework, respectively. Four nitazenes were reported in 2024 through June 1st. Regarding the subjects of the 26 reported nitazene cases, six were Caucasian females (23%), two were African American females (8%), 11 were Caucasian Males (42%), and seven were African American males (27%). Of the reported cases, 35% included subjects aged 30-39.

Polydrug use involving fentanyl, bromazolam, and benzoylecgonine were reported in combination with a nitazene in 81%, 32%, and 23% of cases, respectively. Of the nitazene cases, 19% had six unique analytes present.

Three nitazene case studies were highlighted. A Caucasian male suspected of DUI was arrested and underwent a DRE evaluation, resulting in an opinion of impairment by narcotic analgesics and CNS stimulants. The toxicology analysis resulted in the detection of protonitazene and bromazolam. In another case, a Caucasian male was arrested on suspicion of a DUI due to erratic driving behavior. The toxicology analysis resulted in metonitazene, bromazolam, brorphine, flualprazolam, 6-MAM, morphine, fentanyl, methadone, and xylazine in the blood and/or oral fluid. A third case study of a Caucasian female found hanging from the ceiling fan with previous substance abuse resulted in ten positive analytes in her blood, including metonitazene and etonitazepyne.

Discussion: Over three years, nitazene prevalence increased sevenfold. Fentanyl was found to be the most common analyte reported with a nitazene. N-pyrrolidino protonitazene, N-pyrrolidino metonitazene, and N-desethyl isotonitazene were validated qualitatively by LC/MS/MS. Adding the new targets allows the ADFS Toxicology section to report ten nitazenes qualitatively. Nitazenes' lack of cross-reactivity on the Randox Evidence Analyzer Plus results in challenges when screening for synthetic opioids. As a result, validated nitazenes were added to the QTOF screening database for expanded testing. Due to their emerging prevalence, laboratories should consider adding nitazenes to their testing panel.

P73

A rapid and sensitive UHPLC System-MS/MS method for the analysis of traditional and novel benzodiazepines in Urine

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Introduction: Benzodiazepines are important forensic toxicology drugs which are widely prescribed for neurological and psychiatric disorders but also have a high potential for abuse. Since the mid-2000s, the continued emergence of new designer benzodiazepines, with unknown potency and risks, have raised public health and safety concerns. A particular concern is the growing use of designer benzodiazepines to make counterfeit tablets of commonly prescribed benzodiazepine medication, which can be unknowingly taken.

Objectives: The aim of this study is to develop a comprehensive forensic toxicology method for confirmatory analysis of 26 traditional and designer benzodiazepines via UHPLC MS/MS which includes a rapid and simplified mixed-mode sample preparation method.

Methods: Drug-free urine samples were fortified with traditional (e.g. diazepam) and designer benzodiazepines (e.g. etizolam). A seven-point calibration curve (5-1000 ng/mL) and quality control samples (QCs) at low, medium and high concentrations (15,75,200,750 ng/mL) were prepared. Urine samples (100µL) were added to the wells of a Waters Oasis™ MCX µElution™ plate followed by 100 µL of a solution containing recombinant beta-glucuronidase enzyme, hydrolysis buffer and internal standards and mixed by aspiration. The sample was loaded onto the sorbent bed by vacuum and washed with 200 µL of 80:20 water:methanol. After drying under high vacuum, the samples were eluted using 2 x 25 µL of 50:50 acetonitrile:methanol containing 5% strong ammonia. All samples were diluted with 150 µL of 2% acetonitrile: 1% formic acid prior to analysis.

A UHPLC-MS/MS method was employed using an ACQUITY™ UHPLC™ I-Class PLUS system in conjunction with a Xevo™ TQ-S micro Mass Spectrometer. The MS was operated in electrospray positive ion mode and two multiple rection monitoring (MRM) transitions were monitored for each analyte and a single transition monitored for the deuterated ISTDs. Compounds were separated on an ACQUITY UPLC BEH™ C_{18} Column using a gradient elution profile from 2% mobile phase B (0.1% formic acid in acetonitrile) to 90% over 3.5 minutes. The developed method was assessed for recovery, matrix effects, accuracy, precision and linearity.

Results & Discussion: The panel of 26 benzodiazepine compounds eluted over a 3.5 minute gradient, with retention times from 1.55 to 3.26 minutes, achieving excellent separation and baseline resolution for most compounds. Average extraction recovery was 90.2%, majority within 80-120% for all compounds, except for 8-aminoclonazepam (68%). Matrix effects were evaluated using multiple lots of urine. Ion suppression was observed for most analytes, the absolute matrix effects were 13.7% to -32.9%, and mostly minimized to ±20% after internal standard correction. Calibration curves were prepared and analysed over the range of 5 to 1000ng/mL with regression coefficients (r^2) ≥ 0.99. Coefficients of variation (%CV) were less than 15% for all quantitative compounds tested at 4 concentrations over 5 days. Results for proficiency samples showed excellent agreement with existing procedures.

Conclusion: The described sample preparation method efficiently extracts both traditional and designer benzodiazepines from urine samples. Leveraging the UHPLC-MS/MS method allows rapid analysis of a large panel of benzodiazepines while maintaining required baseline separations. The Xevo TQ-S micro Mass Spectrometer ensures accurate quantitation across a wide dynamic range for all analyzed compounds. This powerful combination results in a rapid, accurate and precise method for the comprehensively analyzing benzodiazepines in urine.

P74

Drug Surveillance in Oral Fluid Samples Collected from New York City Nightclubs

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Introduction: Interest in oral fluid drug testing in forensic toxicology and drug use surveillance is increasing due to ease of collection compared to invasive blood sampling and results being more strongly reflective of recent use compared to urine and hair testing. Oral fluid collection is rapid and simple, allowing for timely field collection and increased likelihood of detecting recent drug use. Consequently, oral fluid is ideal for surveilling recreational drug use and identifying emerging drug trends. There is limited information on broad-spectrum drug screening in oral fluid that includes the identification of traditional drugs, adulterants, and novel psychoactive substances (NPS).

Objectives: This study seeks to gather information on patterns of recreational drug use in New York City (NYC) through peer collection of oral fluid from individuals attending nightclubs. Broad-spectrum toxicology testing is accomplished using liquid chromatography quadrupole time-of-flight mass spectrometry (LC-QTOF-MS) and a semi-quantitative assessment is employed on drugs of interest.

Methods: Oral fluid specimens were collected from consenting individuals attending nightclubs in NYC during 2024. To date, 229 oral fluid samples were collected using Quantisal™ collection devices and shipped weekly to the Center for Forensic Science Research and Education (CFSRE) for analysis. Samples were prepared using two single-step liquid-liquid extractions to recover both basic and acidic drugs. The samples were analyzed using a previously validated broad-spectrum drug-screening method. The instrument used was a Sciex Exion LC coupled to a Sciex X500R LC-QTOF-MS. Data were processed against an in-house database containing more than 1,100 analytes, including traditional drugs, therapeutics, metabolites, adulterants, and NPS. Semi-quantitative analysis was performed using controls prepared at 5, 50, and 500 ng/mL. The semi-quantitative scope included commonly encountered club drugs and other substances: methamphetamine, MDA, MDMA, cocaine, cocaethylene, ketamine, norketamine, dimethylpentylone, alprazolam, flubromazepam, fentanyl, *para*-fluorofentanyl, methylone, tramadol, and *O*-desmethyltramadol. Peak area ratio comparisons to the controls were used to determine approximate drug concentrations using a 5 ng/mL cut-off level.

Results: About half (53%) of the samples received were positive for at least one drug/metabolite. Cannabinoids were most frequently detected (30%), followed by stimulants (24%), hallucinogens (22%), and opioids (2%). Cocaine was frequently detected (21%). Ketamine (18%) and MDMA (9%) were the most frequently detected hallucinogens. Their respective metabolites (cocaethylene, MDA, and norketamine) were commonly identified, alongside the parent drug. Methamphetamine was found in 2% of the samples. Semi-quantitative data for these drugs is shown in Table 1. THC was the most detected cannabinoid (n=72, 30%). LSD was identified in two specimens and psilocin was identified in one. Fentanyl was detected in three of the five opioid positive samples. Quinine (11%) and Lidocaine (10%) were the most common adulterants detected. NPS were detected in 6% of samples and synthetic stimulants were the primary sub-class identified. Methylmethcathinone was the most frequently identified NPS, followed by chloromethcathinone, dimethylpentylone, eutylone, and *para*-fluorofentanyl.

Table 1. Semi-Quantitative Results for Oral Fluid Samples

Compound	N	Mean±Std Dev (ng/mL)	Median (ng/mL)	Range (ng/mL)
Methamphetamine	5	390±530	50	5-1200
MDMA	19	1100±1500	210	5-4700
MDA	9	60±50	50	15-180
Ketamine	43	400±630	70	5-2500
Norketamine	28	90±100	50	5-370
Cocaine	47	660±990	150	5-4500
Cocaethylene	14	100±110	40	5-350

Discussion/Conclusion: As expected, traditionally used club drugs such as cocaine, ketamine, and MDMA were frequently detected along with their metabolites. This is especially true compared to opioids and benzodiazepines. Many club drugs are orally administered via ingesting pills/tablets or smoking, which can cause a greater amount of drug to be present in oral fluid via oral cavity "contamination." Nonetheless, the presence of drugs and relative concentrations in oral fluid is useful in surveilling club drug use and potentially identifying emergent synthetic stimulants and hallucinogens.

P75

Professional Mentoring Program Progress: 2020-2023

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Introduction: The SOFT Professional Mentoring Program (PMP) was designed to provide a platform for toxicologists to interact with others to transfer knowledge and develop long lasting relationships. While each mentoring relationship is unique between pairings, our committee focused on relatable topics in various platforms to provide helpful guidance for both mentors and mentees. In order to have measurable metrics to gauge progress, the program carefully constructed an annual survey for participants to complete after participation in the program. Participant surveys assess committee-defined mentoring benefits, track progress between pairs, establish measurable outcomes, and target program improvements. This feedback is used to evolve the program to meet the needs of the participants to create content of value to the SOFT community as a whole.

Objectives: To annually share program outcomes of mentor/mentee self-reports and communicate the impact of the PMP within SOFT.

Methods: Feedback on participant achievements (goals assessment), and program structure/content (program value assessment) were collected via participant surveys at the end of each mentoring program year. In addition to one-on-one mentoring sessions, the 2023 program offered other activities based on the 2022 participants' feedback, including a kick-off event, webinars, resources emails, book club and a mentoring event at SOFT.

Results: Since the inaugural year in 2020, the program has met the participants' original expectations, and exceeded them in all years: 88% in 2020, 90% in 2021, 92% in 2022 and 89% in 2023. The breakdown of program participation for 2020-2023 is shown in the figure below. For professional goals, the program has shown an impact mainly in 3 areas: SOFT engagement, career advancement, and development of interpersonal/leadership skills. Throughout the years we could see a shift toward the development of interpersonal/leadership skills; according to survey data, the most significant professional outcome was SOFT engagement in 2020 (54%), career advancement in 2021 (64%), career advancement and development of interpersonal/leadership skills in 2022 (52% each), and development of interpersonal/leadership skills in 2023 (67%). In all four years, participants ranked transfer of knowledge and expanding their professional network as the top benefits (>80%) they obtained from the program. Leadership development was also highlighted as a key benefit by more than 80% of participants in 2021 and 2023. Among the different activities organized by the committee from 2021 to 2023, the three most valued (more than 60% high or moderate value) were monthly resources emails in 2021, impostor syndrome webinar in 2022, and resources published in ToxTalk in 2023.

Discussion: Over the four years, participants to the program have increased due to multiple honorable mentions by graduates at various SOFT events. Positive and negative feedback are evaluated consistently by the committee to guarantee successful program events. The importance of keeping the PMP as a structured program allowed for a comparable dataset year over year. The comparison of all data within the year and across years helps with future planning and sets-up new areas of focus for the program. The customary relationship and the confidentiality clause allow an open dialogue between the mentor and mentee. The goal of the mentoring agreement is to provide participants a safe and open space where important interpersonal development can naturally occur. The relationships built and maintained are evidence that the PMP program is and continues to provide leadership development and legacy-building transfer of knowledge within SOFT.

P76

Microsomes and Microchips - Proof of Concept of an Easy-to-use Workflow for Rapid Elucidation of Metabolites for MS-based Screening

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Introduction: Mass spectrometry (MS) is the gold standard for the identification of substances in a standard toxicological analysis. Regardless of whether MS is used for screening or as confirmatory analysis, these methods must be kept constantly updated. However, especially for emerging substances, e.g. the dynamic class of new psychoactive substances (NPS), reference standards for metabolites are usually not commercially available. This is a particular problem for substances whose parent compound is barely detectable or not detectable at all in urine. Insufficient knowledge of metabolism and a lack of spectral information of metabolites may lead to false negative results.

Objectives: Evaluation of a non-targeted workflow using UPLC-timsTOF-MS and MetaboScape® with in silico prediction, in silico fragmentation and collision cross section (CCS) prediction of metabolites using quetiapine as a model substance and subsequent analysis of urine samples.

Methods: Phase I metabolites of quetiapine were generated using an established pooled human liver miscrosomes (pHLM) assay. The analysis was performed with a 20 minute gradient on an Elute UHPLC connected to a timsTOF (Bruker Daltonik). Ionization was performed with a VIP HESI ion source in positive ESI mode. Metabolite prediction was performed with Biotransformer 3.0. Features were extracted in MetaboScape® using the time aligned region complete eXtraction (T-ReX®) 4D algorithm and annotated using data from CCS prediction and in silico fragmentation of predicted drug metabolites. High resolution spectra of known quetiapine metabolites (MMHW library, Wiley-VCH) were used to confirm the results. Twenty urine sample previously found positive for quetiapine were analyzed to compare the relative intensities of metabolites in vitro and in vivo.

Results: This workflow led to the annotation of 18 phase I metabolites in the pHLM assay. Eleven metabolites could be confirmed by library match with the MMHW library. Sulfoxidated, mono- and di-hydroxylated, as well as an N- and an O-dealkylated species were the most intense metabolites found in vitro. Two metabolites (e.g. Quetiapine-M (N-CH2-COOH-sulfoxide) with a modification on the side chain could only be identified by library matching.

Retention time and qualifier ions of all metabolites annotated in MetaboScape were added to the TargetSceener HR database and 20 quetiapine positive samples from forensic casework were either re-analyzed or existing broadband CID (bbCID) data was re-evaluated with the updated method. The N- and O-dealkyl as well as the carboxylated metabolite were the top three most abundant biotransformation products detected in human urine samples.

Discussion: The use of pHLMs, UHPLC-timsToF-MS and a combination of sophisticated software tools to detect metabolites of the model compound quetiapine after biotransformation prediction, CCS prediction and in silico fragmentation of potential metabolites was successfully demonstrated. This workflow enables an easy-to use and rapid detection and annotation of drug metabolites in a single analysis. Results were in good agreement with known data from the literature and spectral libraries.

It is self-evident that in vitro assays using pHLMs cannot fully replicate the metabolism of the human body, so quantitative and qualitative differences between pHLM results and data from human samples are to be expected. Two side chain modificated metabolites could not be annotated by the software tools but only by library matching, showing that the quality of the used biotransformation prediction appears to be one of the main limitations of this workflow. Therefore, this workflow alone is not intended for complete metabolic elucidation of a compound, but as a simple and less time-consuming method to find biomarkers and the necessary feature information to set up an MS-based screening of these metabolites in human urine samples.

Metabolic information obtained by the non-targeted analysis was used to adapt our routine LC-QTOF-MS screening

approach to successfully analyze quetiapine-positive urine samples from routine casework.

P77

Rapid Seized Drug Analysis by RADIAN ASAP MS and Confirmation by High Resolution Mass Spectrometry

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Introduction: Illicit drug use and trafficking causes harm, instability, and violence; the analysis of seized drugs plays a vital role in the effectiveness of national and international programs which aim to control the use, trafficking and distribution of illegal drug substances. The increased number of samples seized and submitted for analysis along with diversity and potential toxicity, places a huge burden on drug control and forensic laboratories to produce reliable results quickly. Consequently, analytical methods that provide fast, dependable results are of interest. Two hundred and twenty-nine suspect materials, confiscated at music events/night-time venues, were supplied by the UK police.

Objectives: The aim of this study was to assess the potential of a compact device based on Atmospheric Solids Analysis Probe-Mass Spectrometry (RADIAN™ ASAP MS), as a simple, rapid screening tool for the detection of drug substances in seized materials and subsequent confirmatory analysis using an established UHPLC-TOF-MS method.

Methods: The seized materials, including pills, capsules and powders, were fully dissolved into 5 mL of methanol, diluted 1:20 with methanol and sampled by dipping a glass capillary prior to analysis by RADIAN ASAP MS. Mass detection was performed using full scan MS (*m/z* 50-650), at 600°C with an analysis time of approximately 30 seconds. Data were acquired simultaneously at four cone voltages (15, 25, 35, 50 V), which generated characteristic precursor and fragment ions. Data were processed using LiveID™ Software and compared with the seized drug spectral library (Waters) for 79 analytes. A minimum average match score of 850 (from a maximum 1000) was used as the reporting criteria for a positive detection.

Confirmatory analysis was performed using the Waters ACQUITY™ UPLC™ I-Class System combined with a Xevo™ G3 QTof Mass Spectrometry (UHPLC-TOF-MS) to assess the performance of the RADIAN ASAP MS. Seized sample stock solutions were diluted, 1:2,000 with 5mM ammonium formate pH 3.0, prior to screening. Chromatographic separation was achieved in 15 min and data were acquired using MS^E acquisition mode. Identification was based on retention time, precursor mass detection, and the presence of at least one diagnostic fragment ion and compared with the Waters toxicology library.

Semi-quantitative analysis was evaluated using the RADIAN ASAP MS on a sub-set of the seized material identified as containing cocaine and MDMA. A solvent calibration series (5 µg/mL – 1000 µg/mL) was prepared using certified reference materials in methanol containing an internal standard (ISTD molsidomine at 25 µg/mL). The seized sample stock solutions were diluted with methanol containing the ISTD. The prepared standards and samples were subsequently analysed following the acquisition procedure previously described.

Results & Discussion: RADIAN ASAP MS analysis of the 229 seized samples led to the detections of one or more compounds, when matched to the seized drug spectral library. The RADIAN ASAP MS demonstrated excellent qualitative agreement with the UHPLC-TOF-MS analysis, confirming the 252 identifications: MDMA (74%), flualprazolam (9.5%), cocaine (7%), caffeine (4%), paracetamol (4%), etizolam (2%) and amphetamine (0.5%) with average match scores ranging from 898 to 993. The UHPLC-TOF-MS confirmatory analysis also detected additional compounds, which is likely due to the expanded UHPLC-TOF-MS library content and increased analytical sensitivity.

Semi-quantitative evaluation on a sub-set of seized samples for cocaine and MDMA, detected analyte concentrations ranging between 78mg to 170mg for cocaine, and 18mg to 50mg for MDMA.

Conclusion: RADIAN ASAP MS is a compact device which provides a rapid and easy-to-use screen that can identify single or multiple components in seized materials. The simple workflow has demonstrated consistency and confidence in identification of compounds across pills, powders and capsular seized samples.

P78

UHPLC-TOF-MSE Analysis of a Cohort of Patient Urine Samples from Drug Rehabilitation Centre's and an Emergency Department

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Introduction: Over the past two decades, the drug market has witnessed a surge in the availability of new psychoactive substances (NPS) and designer drugs. These substances are designed to mimic the effects of controlled drugs while often bypassing international drug control regulations. As a result, toxicology laboratories frequently encounter the need for comprehensive screening techniques to analyze complex biological matrices. The purpose of this research was to increase our understanding of emerging drug trends, and to highlight the importance of staying ahead of the ever-evolving landscape of illicit substances. For this study, urine samples were anonymously acquired from drug rehabilitation centres, and an emergency department in the UK, with the latter suspected of acute drug toxicity.

Objectives: Our primary objective was to identify and characterize the urine samples based on a verified broad toxicology screen which facilitated the unambiguous identification of a targeted list of drugs. The secondary objective, and more importantly, our investigation extended beyond known identifications, exploring the possibility of 'what else is out there', leveraging the discovery toolset within waters_connect™ Software to characterize the unknown peaks observed during the screen. Data were acquired using a UHPLC-TOF-MS, providing accurate mass information for the precursor and associated fragment ions.

Methods: The Waters ACQUITY™ UPLC™ I-Class System combined with a Xevo™ G3 QTof Mass Spectrometry (UHPLC-TOF-MS) was used for sample characterization. Samples were diluted, 1:5 with 5mM ammonium formate pH 3.0 or with 0.001% formic acid in water prior to screening in positive or negative ionization mode, respectively, with the UHPLC-TOF-MS. Chromatographic separation was achieved in 15 min (positive mode) and 7.50 min (negative mode) and data were acquired using MS^E acquisition mode. Identification was based on retention time (±0.35 min of reference retention time), detection of a precursor mass, and the presence of at least one diagnostic fragment ion. Data were compared with an established Waters toxicology library based on retention time and accurate mass fragment data for over 2000 analytes.

Results: A number of parent drugs together with their metabolites were detected. A percentage of the Waters toxicology library were evaluated for limit of detection, with a minimum concentration of 50 ng/mL attained, based on a precursor response of at least 10,000 counts. These were categorised into: cocaines, opiates/opioids, benzodiazepines/z-compounds, antidepressants, stimulants, cannabinoids, anticonvulsants, antipsychotics and an all-encompassing category of others, which included ß-blockers, decongestants and vasodilators. Subsequent elucidation of the unknown peaks identified a number of designer substances in the form of nitazenes: protonitazene, metonitazene, etodesnitazene, isotonitazene and isotodesnitazene. Several potential drug adulterants were also detected as well as prescription/over-the-counter medications.

Discussion: The TOF-MSE analysis revealed valuable insights into the composition of diluted urine samples, detecting drugs and their metabolites. This technique excels in screening for novel drugs, even when standard reference materials are unavailable. The power of structural elucidation was evident in identifying new and emerging classes of compounds like the nitazenes.

In summary, TOF-MSE represents a robust approach for comprehensive drug screening, providing essential information for clinical and forensic applications. As the landscape of illicit substances continues to evolve, methodologies like TOF-MSE play a crucial role in staying ahead of emerging compounds.

P79

Unveiling Metabolite Features of Synthetic Cannabinoids Suitable for Urine Screening - T-ReXing UHPLC-timsTOF-MS Data of pHLM Assays

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Introduction: Since urine is a preferred screening matrix for toxicological analysis, MS-based methods need to contain metabolites of forensically relevant substances. Unfortunately, the availability of reference standards for drug metabolites, especially for new psychoactive substances (NPS) like synthetic cannabinoids (SCs), is limited. To prove consumption of new SCs by urine analysis, biomarkers have to be rapidly added to the respective MS methods. If parent compounds are available, pooled human liver microsome (pHLM) assays are an easy-to-use method for generating phase I metabolites. Trapped ion mobility time-of-flight mass spectrometry (tims-TOF) combined with sophisticated software tools helps to predict and annotate features of potential metabolites.

Objectives: A non-targeted workflow using UPLC-timsTOF-MS and MetaboScape®, incorporating *in silico* prediction of metabolites, collision cross section (CCS) prediction and *in silico* fragmentation was used to elucidate metabolic features of three prevalent SCs for subsequent urine screening.

Methods: Phase I metabolites of ADB-BUTINACA, MDMB-4en-PINACA and MDMB-BUTINACA (c =10 µg/mL) were generated using an established pHLM assay (incubation time:0.5 h at 37°C). The reaction was quenched using ice-cold acetonitrile and ammonium formate (10 M). The residue of the liquid phase was reconstituted in 50 µl LC eluent. Analysis was performed on an Elute UHPLC connected to a Bruker timsTOF Pro 2 equipped with a VIP-HESI (Vacuum Insulated Probe Heated Electrospray Ionization) ion source. BioTransformer (www.biomarker.ca) and GLORYx (https://nerdd.univie.ac.at/gloryx/) were used for metabolite prediction. For feature extraction the T-ReX® (Time aligned Region complete eXtraction) 4D algorithm, CCS prediction and *in silico* fragmentation of the predicted metabolites in MetaboScape® were used. SC positive urine samples were re-acquired in PASEF® (parallel accumulation serial fragmentation) and bbCID (broadband collision-induced dissociation) mode to identify *in vivo* metabolites. Retention time, CCS values and qualifier ions found in this step were added to the TargetScreener HR database.

Results: For ADB-BUTINACA, multiple monohydroxylations and primary amide hydrolysis were predicted and successfully annotated. However, the most abundant *in vivo* metabolite according to literature, the dihydrodiole formation at the indazole scaffold, was not predicted *in silico*.

For MDMB-4en-PINACA, the highly abundant ester hydrolysis metabolite as well as dihydrodiole formation and monohydroxylation at the *N*-pentenyl side chain were predicted and successfully annotated. In addition, several monohydroxylated metabolites could be annotated in the majority of urine samples.

For the novel SC MDMB-BUTINACA, an ester hydrolysis metabolite and the combination of hydrolysis and hydroxylation were predicted and successfully annotated. These non-specific metabolites are also formed after ingestion of ADB-BUTINACA but to a much lesser extent. In addition, compound specific monohydroxylated biomarkers could be predicted and successfully annotated in pHLM extracts and some urine samples. Inter-individual differences in enzyme activity or different time periods between uptake and urine sampling might be an explanation for this.

Discussion: MetaboScape® was able to detect and annotate high abundant and compound specific metabolites. Although, the tims data has shown, there are several metabolites of the same sum formula, the site of biotransformation could often not been determined. For the evaluation of biomarkers for MS screening in urine, this is considered to be negligible. Qualitative findings from pHLM and urine samples from case work were largely congruent, quantitative differences were to be expected.

However, the *in silico* prediction of metabolites is the bottleneck of this approach, e.g. the dihydrodiole metabolite of ADB-BUTINACA could not be predicted by either of the two algorhythms. Some metabolites required further manual

processing steps to predict them in silico. This might become more challenging for SCs with uncommon substituents like *N*-trimethylpropylsilyl sidechains.

This untargeted workflow provides a sensitive detection and confident annotation/identification of SC biomarkers. Due to the combination of *in silico* prediction and data from pHLM or real samples, respectively, features of SC metabolites can be added to an MS screening without having the respective standard on hand.

P80

Evaluating Drug Positivity at BACs of >0.10 g/100 mL Across Five States

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Introduction: Stop-limit testing is the practice of choosing whether or not to perform drug testing based on an administratively determined alcohol concentration. This practice is often justified when there is a high blood alcohol concentration (BAC) that can explain the impairment and/or due to the limited resources and budget that toxicology laboratories face. Ongoing public safety and health concerns with respect to impaired driving (DUID) however, benefit from as much information as possible being gathered about impaired drivers' alcohol and drug use. With stop-limit testing, important drug positivity information is ignored and results in underreporting of drug prevalence in impaired driving cases.

Objective: The objective of this study was to evaluate drug positivity at a common BAC stop-limit threshold for five different states.

Methods: Blood samples submitted in suspected DUID cases were retested using an extensive library of legacy and novel drugs. Samples were received from five different states including Pennsylvania, Wisconsin, Montana, Missouri, and Ohio. All states except Wisconsin sent samples at random, regardless of the laboratory's results. Wisconsin only submitted cases with a BAC >0.10g/100mL, as the current policy is no drug testing is pursued when a BAC is greater than 0.10g/100mL. All samples underwent alcohol testing at the originating laboratory, and those results were provided with the samples. Once received, all samples underwent testing for basic drugs and gabapentin using liquid chromatography/mass spectrometry quadrupole time-of-flight (LC-QTOF). All samples were also evaluated for THC and its metabolites. Pennsylvania samples underwent testing for synthetic cannabinoids and due to the low positivity for these analytes was not pursued for samples from other states. Drug test results were evaluated for the National Safety Council's Tier I and/or Tier II drugs relative to BAC threshold of >0.10g/100mL.

Results: In total 1,025 samples were analyzed as part of the study. There were 516 samples from Pennsylvania, 193 from Missouri, 116 from Ohio, 100 from Wisconsin, and 100 from Montana. All states showed positivity for Tier I and/or Tier II drugs at a BAC level >0.10 g/100mL. Positivity for Tier I drugs-only was highest, followed by Tier I and Tier II drugs in combination, and Tier II drugs-only as shown in Table 1

Table 1. Percentage of cases with a BAC >0.10g/100mL by state that were positive for other drugs.

	PA	MO	MT	OH	WI
Tier I only	16% THC-16% Methamphetamine-1.0%	29% THC-32% Methamphetamine-3.0%	42% THC-42% Amphetamine-2.0%	22% THC-26% Methamphetamine-3.4%	57% THC-61% Methamphetamine-5%
Tier II only	2.5%	4.1%	2.0%	2.5%	4.0%
Tier I and Tier II	3.8%	6.2%	4.0%	6.9%	9.0%
Tier I, Tier II, or Combination	23%	39%	48%	31%	70%

Discussion: The findings show Tier I and/or Tier II drugs were present in greater than 20% of cases across all states at a BAC >0.10g/100mL. Of significance, the vast majority of cases with drug findings were positive for a Tier I drug, which are categorized as drugs known to cause impairing effects. In many of the samples received, drug testing had not been performed due to stop-limit testing policies leading to underreporting of DUID positivity. In the data obtained, THC and methamphetamine were the most commonly encountered Tier I drugs. Frequently detected Tier II drugs included gabapentin, diphenhydramine, and hydroxyzine.

The data here demonstrates that even cases with high BACs should be analyzed for other drugs in order to obtain more insight into drug use in impaired driving cases. In order to generate data-driven solutions from both a public health and safety perspective, drug testing in suspected impaired driving cases that includes the National Safety Council's Alcohol Drug and Impairment Division's Tier I recommendations is a best practice.

P81

Investigation of the effect of three solvents on the quantitative results for 7-Aminoclonazepam.

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Introduction: 7-aminoclonazepam, the major active metabolite of clonazepam, is often tested for in forensic laboratories to monitor for clonazepam use. It can have sedative effects and forensic toxicologists are often asked to discuss these effects in court. When interpreting cases involving 7-aminoclonazepam, it is imperative to have accurate quantitative results. 7-aminoclonazepam certified reference material is prepared by the manufacturer and shipped in acetonitrile. Due to this, it is expected that calibration curves prepared in acetonitrile will generate the most accurate quantitative values for both quality controls and casework.

Objective: The objective of this study was to determine whether using calibrators prepared in different solvents created a significant difference in quantitative results for 7-aminoclonazepam.

Methods: Calibrator stock solutions were prepared in both methanol and acetonitrile. An additional calibrator solution prepared in water was also included in the study for reference. Calibrators prepared using each solvent were spiked into an eight-point whole blood calibration curve ranging from 5 – 1000 ng/mL. Externally prepared methanolic quality control solutions were spiked into whole blood and urine. Calibrators, controls, and case specimen whole blood samples were extracted with a solid phase extraction procedure followed by quantitative analysis via LC/MS/MS.

Results: Samples were quantified using the calibration curves created from each solvent over eleven runs. All calibration curves utilized linear 1/x weighting not forced through zero and had r^2 values ≥ 0.990. The percent difference from the target values of calibrators and controls were compared across solvent types. When utilizing a matched solvent, the methanolic calibrator and quality control percent difference averages from the targets were all <10%. When utilizing a non-matched calibration solvent, the average percent differences from the target for the methanolic quality controls were >44%, which was outside the acceptable range. It appeared that the acetonitrile and water calibration curves produced similar responses, but quality controls and case samples were both higher than the concentrations achieved when utilizing a methanolic calibration curve.

Due to the responses seen across solvents, in-house acetonitrile and water quality control solutions were prepared. When utilizing a solvent matched calibration curve, the average percent difference of these quality controls from their target values was <20%.

A 2-tailed paired student's t-test with a significance value of 0.05 was also used to compare the data. All t-test results for the calibrator and quality control comparisons listed above gave values < 0.05. Case specimens within the limits of quantitation were compared and all three comparisons gave values < 0.05, indicating that the differences were significant.

Discussion: When comparing case specimen results, the methanolic calibrator solution produced the lowest quantitative values, while the water calibrator gave the highest values. The 7-aminoclonazepam certified reference material utilized for these experiments is shipped from the manufacturer in acetonitrile. Due to this, it was expected that a calibration curve created using acetonitrile would produce the most accurate quantitative values. There was great variability in the obtained concentrations, depending on which solvent was used for the 7-aminoclonazepam addition to the matrix when creating a calibration curve. It was also noted that when utilizing a methanolic control, they quantified higher than the acceptable range on both the acetonitrile and water curves. It appears that the control only passes the acceptability criteria when matched with the same solvent. It was also noted that case samples with concentrations on the lower end of the linear range could potentially be reported as negative when utilizing a methanolic curve but would be reportable when utilizing an acetonitrile curve. This may impact the interpretation of the case.

P82

Intelligent Reflex Automatic Worklist Intervention for Toxicological Drug Screening by High-Resolution LC/Q-TOF

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Introduction: Data-independent acquisition (DIA) is advantageous in large library screening methods for drug analysis. Using LC/Q-TOF, information can be gathered on targets and suspects simultaneously and further, older sample data files can easily be re-investigated for the existence of emerging analytes of interest. Screening for a large range of analytes at many different concentration levels can, however, result in the need for manual evaluation and possible sample reinjection. Intelligent reflex workflows with MassHunter Acquisition have been developed to increase instrument up time and reduce the need for manual worklist setup. Outlined herein is a demonstration of two intelligent reflex workflows performed using the Revident LC/Q-TOF. Evaluation of drug analytes over a range of concentrations in solvent, plasma and urine is demonstrated using workflows to automatically manage carryover and detection of values above the calibration curve with adjusted sample reinjections during real-time analyses.

Objectives: To demonstrate the capabilities of new intelligent reflex workflows for drug screening using a new software tool for fast reflexive worklist management for carryover and above calibration curve detection through automated worklist appending and injection volume reduction.

Methods: Urine and plasma samples prepared by dilution and EMR-Lipid extraction, respectively, were spiked at 8 calibration levels from 1 to 100 ng/mL with 32 scheduled drugs and 16 heavy labeled analytes at 50 ng/mL. An 11-minute reverse phase LC gradient method was optimized on a 100 mm Poroshell 120 EC-C18 column. Non-targeted acquisition was carried out using All Ions methodology at collision energies 0, 20 and 40 eV. MassHunter Quantitation methods were built using library MS/MS spectra for comparison of molecular ion and fragment ions and curated further with retention times specific to the chromatography gradient. Intelligent reflex was enabled in the worklist during data acquisition to address carryover and samples detected above highest calibration levels in real time.

Results: All Ions acquisition was used for non-targeted analysis of spiked calibration curves between 1 and 100 ng/mL (n=4), and results will be shown for plasma and urine matrix. Initial analysis showed, for the 32 compounds of interest the screening method designated all compounds as identified at the 5 ng/mL level with most identified < 5 ng/mL. The screening method utilizes mass accuracy, retention time, mass match score, and number of verified ions. Most calibration curves showed good linearity over the entire concentration range, with correlations $R^2 > 0.99$. Precision of 95% of the response RSDs for each calibration level were below 10%, and mass accuracies were within ±2 ppm for most compounds.

Discussion: The calibration curve reflexive logic was tested by the worklist integration of a sample over-spiked with 6 compounds at 500 ng/mL. Upon detection of signal over the calibration levels in the associated MassHunter Quantitation method, the sample was reinjected, and injection volume was reduced from 4 uL to 0.5 uL, placing the new data within the calibration range. Carryover management was engaged by setting a blank concentration threshold of 1 ng/mL, and upon detection of carryover in the blank by the instrument, an additional blank injection was inserted into the worklist and replicated until a workflow limit or no carryover detection. Intelligent reflex methodology can be utilized for non-targeted drug screening workflows, increasing instrument up time and reducing the requirement for manual interpretation between initial injection and subsequent reinjections. Shown herein, this can be beneficial for automatically handling samples with results above calibration curve levels and those showing carryover in integrated blanks. In combination with succinct LC/Q-TOF data analysis supported by the LC Screener tool, complex analysis can be simplified yielding clear and accurate results.

P83

Non-Nicotine E-Cigarette Products Branded as Personal Diffusers

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Introduction: Traditional electronic cigarette (e-cig) liquids (e-liquids) are usually composed of a mix of propylene glycol (PG) and vegetable glycerin (VG), combined with flavorings, chemicals of unknown purpose, and a pharmacologically active compound, most commonly nicotine. New vaping products containing an essential oil/blend as the primary ingredient(s) have emerged in the marketplace in recent years. They are sold as non-nicotine "diffusers" or "personal aromatherapy" products, and marketed as a health-focused lifestyle choice. Some of these products are similar to traditional e-cig and use e-liquids. Little about their composition has been published. These products are unregulated with little to no quality assurance and safety protections. The essential oils used in these diffusers have been implicated in allergic reactions, inflammation, and skin irritation.

Objectives: To assess the safety of the chemical constituents in personal aroma diffusers.

Methods: Füm, MonQ, Ripple+, and LUVVair diffusers were purchased from online retailers. Each diffuser was equipped with either a liquid chamber or saturated solid core containing the mixture to be inhaled. The liquid or core was extracted with methanol for chemical characterization by a general unknown method using gas chromatography mass spectrometry, and analyzed for volatiles (ethanol, acetone, isopropanol) using headspace gas chromatography with a flame ionization detection. Product information was pulled from vendor websites The Globally Harmonized System of Classification and Labeling of Chemicals (GHS) was used to assess each identified compound.

Results: Acetone, ethanol, and isopropanol, were present in Ripple+ and MonQ diffusers. Both MonQ and Ripple+ diffusers contained vegetable glycerin (VG), propylene glycol (PG), and other extract flavorings and volatiles. While only VG and PG were present in the LUVVair and Füm diffusers. All products had evidence that the essential oils were included on the package materials or labeling. Many of the products contained additional unlabeled ingredients. All products indicating melatonin, collagen, or CBD on the label contained these compounds.

Discussion: The adoption of the consumer-friendly term "diffuser", as opposed to "e-cig", is deceptive. Some of the diffusers indicated uses by inhalation through the mouth and exhaling through the nose. Inhaling essential oils or extracts into lungs can pose significant health and safety risks. According to GHS rankings, some chemicals identified in the products are a health hazard when inhaled. The impact of inhaling concentrated essential oils, as opposed to a diluted diffusion via a room diffuser, has not been studied. Incomplete ingredient labeling highlights a lack of regulatory oversight. The presence of potentially harmful essential oils, solvents, and other ingredients in diffusers underscores the potential dangers of these diffuser products.

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Ultra-fast Online SPE LC/MS/MS for the Simultaneous Analysis of Drugs in Human Plasma Using the Multiplexed 4-channel System

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Introduction: The Multiplexed 4-channel LC/MS/MS system uses up to 4 multiple, alternating sample introduction streams to keep a single mass spectrometer working continuously. Online solid phase extraction (SPE) automatically reduces matrix effects in LC/MS/MS and enriches the analyte to increase reproducibility and sensitivity without complicated sample pretreatment. Combining these technologies, multiplexed 4-channel online SPE LC/MS/MS system is expected to maximize ruggedness and robustness while increasing sample throughput. The object of this study is establishment of rapid online SPE LC/MS/MS method for drugs in human plasma coupled to high-throughput multiplexed 4-channel system

Methods: Amiodarone, Amphetamine, Codeine, Diazepam, Doxepin, Haloperidol, Oxazepam, Methamphetamine, Phentermine, Fentanyl, and 3,4-MDMA were purchased from Restek. Fluoxetine, Carbamazepine, Meprobamate, and Naproxen were purchased from Cerilliant. Commercially available human plasma was purchased from Golden West Diagnostics, LLC. Plasma (500 µL) was mixed with acetonitrile (1,500 µL), vortexed and then centrifuged. 15 drugs were spiked into methanol and used as neat STD solution. The neat STD solution (100 µL) and the treated supernatant (900 µL) were mixed and used as post-spiked plasma samples. A sample was transferred into the LC vial and set in autosampler. The newly designed online SPE column was used to achieve ultra-fast Online SPE and chromatographic separation was performed with Shim-pack Scepter C18-120 (3 µm, 2.1 x 50 mml.).

Results: 15 drugs which have different hydrophobicity from each other were selected to evaluate online extraction conditions The parameters, such as initial solvent concentration, flow rate, loading time and elution solvent on online SPE step were optimized. Additionally, the transition of target drugs from online SPE column to analytical column was adjusted through confluent flow rate of online SPE and analytical pumps. Finally, the chromatographic selectivity was demonstrated through the baseline resolution of isobaric drugs such as Methamphetamine and Phentermine. Resulting from the above chromatographic optimization, newly designed online SPE column demonstrated mean 90% recoveries which was calculated with only analytical column and online SPE LC/MS/MS.

The carryover in Online SPE column was eliminated by flushing with organic solvent during its idle time. Each flow path was independent and there was enough time to wash the line right after the analysis.

Based on the chromatographic optimization and wash techniques, the measurement time in single stream was 7.3 minutes including automated sample clean-up using online SPE. The multiplexed 4-channel online SPE LC/MS/MS system delivered 4.4 min/measurement which was higher sample throughput than single stream. Compared with single and multiplexing system, throughput increased 36%. It showed that the multiplexing system maximize the productivity.

All the compounds showed excellent quantitative results. For example, the calibration range of Meprobamate which has low-hydrophobicity was 0.1 to 50 ng/mL in post-spiked plasma samples and the R2 was 0.998. the calibration range of Amiodarone which has high-hydrophobicity was 0.01 to 50 ng/mL in post-spiked plasma samples and the R2 was 0.999. As a result of this study, the established method for an ultra-fast online SPE accomplished adequate performance to achieve quantitative analysis using the high-throughput 4-channel online SPE LC/MS/MS.

Discussion: The developed online SPE LC/MS/MS with a multiplexed 4-channel system allows drugs which have wide hydrophobicity to be screened with higher throughput in plasma quantitatively.

P85

Acetyl Fentanyl Detection in Umbilical Cord Tissue: Prevalence of Prenatal Exposure and Co-Exposure with Other Substances in a High-Risk Population

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Introduction: In recent years, the abuse potential of various fentanyl analogs has become a growing concern. Acetyl fentanyl was classified as a Schedule I drug by the DEA in 2015 due to its dangerous recreational use (Finkelstein et al, 2019). The substance has been linked to fatal outcomes and is often underreported. Both acetyl fentanyl and its metabolite, acetyl norfentanyl, have been detected in numerous forensic cases since 2013 (DEA, 2013). While acetyl fentanyl has been found in various biological samples, there is limited research on the prevalence of prenatal exposure.

Objectives: Our study aimed to investigate the presence of acetyl fentanyl and its metabolite in umbilical cord tissue from a high-risk population, utilizing data collected at a national reference laboratory.

Methods: A secondary analysis was conducted using data from umbilical cord tissue samples received at a national reference laboratory between November 2022 and November 2023 for routine forensic testing. Co-exposure to other substances was included where available based on the requested drug panel. Initial testing was performed using Enzyme-Linked Immunosorbent Assay (ELISA), with presumptive positive samples confirmed using Liquid Chromatography Tandem Mass Spectrometry (LC-MS/MS) in positive ionization mode.

Results: During the one-year period from November 2022 to November 2023, 1770 specimens initially tested presumptive positive for fentanyl using a cutoff of 200 pg/g. Among these, 209 samples confirmed positive for acetyl fentanyl or its metabolite acetyl norfentanyl. LLOQ for acetyl fentanyl as well as acetyl norfentanyl is 80 pg/g. The concentrations of acetyl fentanyl ranged from 80 to 38,186 pg/g with a median of 162 pg/g. In comparison, acetyl norfentanyl concentrations ranged from 82 to 21,440 pg/g with a median of 364 pg/g. Additionally, other commonly abused drugs were detected in conjunction with the fentanyl analogs. Specifically, 97 samples tested positive for amphetamine, 111 for methamphetamine, 85 for benzoylecgonine, 58 for opiates (26 for 6-acetylmorphine), 52 for methadone, 29 for carboxy-delta-9THC, 14 for gabapentin, 7 for buprenorphine, and 6 for benzodiazepines. It is necessary to note that fentanyl and/or its metabolite norfentanyl were present in all 209 samples.

Discussion: Acetyl fentanyl and/or its metabolite was detected in 11.8% of umbilical cord tissue samples that tested positive for fentanyl during the study period. Co-exposure with other substances was also significant, as 92% of acetyl fentanyl positives showed the presence of additional substances other than fentanyl. The detection of fentanyl alone in umbilical cord tissue can complicate the interpretation of toxicological findings where fentanyl was administered to the mother during delivery. However, another retrospective study showed fentanyl was not found in detectable levels after administration during labor and delivery where illicit drug use was not suspected (Jones et al 2020). In contrast, the presence of acetyl fentanyl in umbilical cord tissue definitively indicates maternal illicit drug use. Previous research has indicated that acetyl fentanyl may be present in biological samples without the simultaneous detection of fentanyl. This study only included specimens that initially screened positive for fentanyl. Further studies are warranted to explore the cross-reactivity of fentanyl analogs in immunoassay screens, establish appropriate analysis cutoffs, and determine the prevalence of acetyl fentanyl in the absence of other substances.

P86

Validation of ELISA Kits for Oral Fluid Screening

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Introduction: The National Safety Council (NSC) has a mission to help make workplaces and roadways safer. As part of that mission, the NSC formed the Alcohol, Drugs, and Impairment Division (ADID), which is composed of forensic toxicologists, pharmacologists, psychologists, law enforcement officials, and a host of other professionals who share this common goal and work to achieve recommendations for standardization of drug testing.

Oral-fluid drug testing is an area that is becoming increasingly important. In 2013, it was added to the recommendations as a matrix option, and additional iterations support the efficacy of oral fluid. It is also becoming a more popular choice because of low cost, ease of use, and its ability to identify recent drug use.

A study was conducted to evaluate the performance of Neogen's ELISA kits to determine if they meet the recommendations[1] for DUID oral-fluid testing. The panel includes: (-)-Δ9-THC, Buprenorphine, Zolpidem, Cocaine/BEG, Tramadol, Carisoprodol, Opiates (Morphine), Oxycodone, Amphetamine, Methamphetamine, MDA, MDMA, Fentanyl and Benzodiazepines.

Objectives: To evaluate the performance of Neogen's ELISA kits at the recommended screening cutoff concentrations in diluted oral fluid and evaluate the precision at each drug's cutoff concentration using a DYNEX® DSX® ELISA System.

Methods: Each drug analyte or drug group was tested using its corresponding ELISA kit. Testing included dose response curve analysis to determine if the assay adequately detected the drug at the recommended screening cutoff concentration. Precision was also tested at the specified cutoff concentration using the DSX, and a limited cross-reactivity confirmation for each kit was performed. All testing was conducted with oral fluid diluted in NeoSal® Buffer.

Results: The table below shows the Neogen kits that met the recommended screening cutoff concentration with their respective IC50, %B/B0 and coefficient of variation (CV):

Target Analyte	Assay Name	Neat Screening Cutoff (ng/mL)	IC50 (ng/mL)	%B/B0 at Screening Cutoff	CV at Screening Cutoff
(-)-Δ9 THC	THC Oral Fluid (OF)	4	0.80	43	1.81
Amphetamine	Amphetamine Specific-2	20	3.03	43.2	4.52
MDA	Amphetamine Specific-2	20	1.36	37.2	4.7
Benzoylecgo-nine	Cocaine/BZE OF	15	1.94	39.7	6.5
Carisoprodol	Carisoprodol	500	65.9	41.9	6.3
Morphine	Opiates Group OF	30	6.20	47.2	4.1
Buprenorphine	Buprenorphine	1	0.39	64	1.9
Fentanyl	Fentanyl	1	0.18	44.5	2.1
Methamphet-amine	Methamphetamine/MDMA OF	20	8.41	61.3	2.4
MDMA	Methamphetamine/MDMA OF	20	11.1	64.5	0.57
Oxazepam	Benzodiazepine Group	5	1.75	54.7	3
Oxazepam	Benzodiazepine Group Ultra	5	1.84	61.2	3
Oxycodone	Oxycodone/Oxymorphone	30	1.57	53.2	3.7
Methadone	Methadone/LAAM	20	9.57	64	2.06

Conclusion: The majority of Neogen's ELISA kits evaluated detected the target analytes reproducibly in oral fluid at the recommended screening cutoff concentrations. The use of Oral Fluid Buffer was not optimal with the Zolpidem and Tramadol ELISA kits. Additional evaluation was not completed for Zolpidem and Tramadol. Other commercial oral fluid buffers are available but would need to be evaluated to determine compatibility.

[1]Reference: Amanda L. D'Orazio et al., "Recommendations for Toxicological Investigation of Drug-Impaired Driving and Motor Vehicle Fatalities — 2021 Update," Journal of Analytical Toxicology 45, no. 6 (July 2021): 533.

P87

Comparative Analysis of Oral Fluid Drug Testing Methods: Sensitivity and Specificity Insights for 6-Acetylmorphine and Fentanyl

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Introduction: Oral fluid sample testing has become an important matrix for drugs of abuse testing due to its less invasive and observable sample collection. Both the Alcohol, Drugs and Impairment Division (ADID) of the National Safety Council (NSC) and Substance Abuse and Mental Health Services Administration (SAMHSA) have published guidance documents listing recommended oral fluid screening cutoff concentrations for several drugs of abuse. One method for screening oral fluid samples is by ELISA assays. They are a reliable, low-cost method for screening a large volume of samples in a short amount of time.

Objectives: This study of diluted oral fluid samples evaluates the agreement of two Neogen ELISA kits with previously confirmed negative or positive LC/MS/MS results. Further, this study examines the screening cutoff concentrations recommended by ADID (NCS) or SAMHSA Oral Fluid Mandatory Guidelines (OFMG).

Methods: A total of 200 de-identified human oral fluid samples, that were tested by LC/MS/MS (LOD=0.05 ng/mL, LOQ=0.1ng/mL) and identified as positive or negative per analyte, were purchased. These samples were then evaluated for presence of 6-Acetylmorphine and Fentanyl using ELISA kits. Controls were made at a 1:4 diluted concentration for the ADID (NSC) (1 ng/mL for Fentanyl, neat concentration) or SAMHSA OFMG (4 ng/mL for 6-Acetylmorphine, neat concentration) screening cutoff concentrations and at -50% and +100% of the cutoff concentration. Each sample's absorbance value was compared to absorbance value at each drug's recommended cutoff concentration. If the sample's absorbance value was greater than the cutoff concentration, the sample was identified as negative. If the absorbance value was less than that of the cutoff concentration, the sample was identified as positive.

Results: The 6-Acetylmorphine ELISA had 77 samples with a positive screening result and 123 samples with a negative screening result. LC/MS/MS results had 67 samples with a positive result and 133 samples with a negative screening result. Comparison of the screening result to the confirmation results yields a 98.5% Sensitivity and 91.7% Specificity. The Fentanyl ELISA had 82 samples with a positive screening result and 118 samples with a negative screening result. LC/MS/MS results had 82 samples with a positive result and 118 samples with a negative screening result. Comparison of the screening results to the confirmation results yields 100% Sensitivity and 100% Specificity.

Discussion: The Fentanyl ELISA screening results were in perfect agreement with the LC/MS/MS results. The 6-Acetylmorphine ELISA screening had a high sensitivity term but reduced specificity outcome. The samples that were false positive in the 6-Acetylmorphine ELISA could not be rationalized due to known assay cross-reactivity profile. The high sensitivity term for 6-Acetylmorphine ELISA should allow positive samples to be identified but may require further optimization to improve the specificity term. Neogen's ELISA kits can be used for rapid and reliable screening of Fentanyl and 6-Acetylmorphine positive samples at ADID and SAMHSA recommended cutoff concentrations.

P88

Mass spectrometric screening for drugs in urine in the clinical toxicology laboratory

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Introduction: In clinical toxicology, drug screening may be used in the diagnosis and treatment of an acutely ill patient to monitor compliance with medication or abstinence from illicit drug use. Urine immunoassays (IAs) are simple tests that are easy to perform, may be automated, and provide a rapid turnaround time (TAT). However, they may lack specificity, and FDA-approved tests are generally limited to illicit drugs, benzodiazepines, and opioid medications.

An alternative approach is to use mass spectrometry as a screening tool. Challenges with this technique include the time required for sample preparation, instrument run time, and review of results, which may not be amenable to automation.

Objective: The objective of the study was: 1. To identify the drugs most often observed in specimens submitted to a clinical toxicology laboratory for testing by gas chromatography-mass spectrometry (GC/MS). 2. To evaluate if those drugs can be detected by current IAs available in our laboratory.

Methods: We retrospectively analyzed results from deidentified, consecutive urine specimens that had been submitted to Quest Diagnostics for comprehensive urine drug screening by GC/MS in 2023. This qualitative CLIA-validated, laboratory-developed method tested for the presence of 94 drugs and metabolites. Specimens came from New England medical centers, including acute/urgent care centers, community health centers, and primary care providers. Ordering clinicians could request either “stat” (4-hour TAT) or routine (24-hour TAT) testing.

Results: A total of 2,096 specimens were identified. “Stat” testing was requested for 821 (39.2%) specimens, all of which were from hospital settings. A slight majority of specimens were obtained from female patients (1,061 [50.6%]), and patient sex was unknown for 4 specimens. Patient age ranged from <1 to 97 years (mean, 37; median, 37), with 331 (15.8%) specimens from individuals less than 1 year of age. A low number of specimens (90 [4.3%]) were drug-free including caffeine, cotinine and/or nicotine, which were found in 557 specimens (27.8%). 115 of which were from children ≤12 years old. Other drugs or metabolites found, in order of prevalence, were lidocaine/metabolite (369 [18.4% of positives]), diphenhydramine (254 [12.7%]), cocaine/benzoylecgonine/ecgonine methyl ester (190 [9.5%]), acetaminophen (185 [9.2%]), and fentanyl (166 [8.3%]). The most prevalent drugs or metabolites from other classes were diazepam/nordiazepam (73 [3.6%], benzodiazepines), diphenhydramine (antihistamines), quetiapine/nor quetiapine (78 [3.9%], antipsychotics), and bupropion/metabolite (104 [5.2%], antidepressants). No specimens contained 6-acetylmorphine, meperidine, phencyclidine, or strychnine.

The number of specimens with 1 drug, excluding caffeine, cotinine, and nicotine, was 600 (28.6%), the number with 2 drugs was 392 (18.7%), the number with 3 drugs was 258 (12.3%), and the number with >3 drugs was 288 (13.7%). The greatest number of drugs found in a specimen was 11, which included illicit and prescription drugs.

A number of specimens (69.3% of positive specimens) contained drugs that would not have been detected by the IAs available in our laboratory. These include anticonvulsants such as carbamazepine, gabapentin, and lamotrigine; tricyclic antidepressants; antipsychotics such as quetiapine and olanzapine; zolpidem; and over-the-counter drugs.

Discussion: The majority (96%) of specimens contained at least 1 drug, including the most prevalent significant drugs lidocaine/metabolite, and diphenhydramine. The results may assist the clinician in understanding the condition of a patient to improve care in a timely manner. In addition, a significant proportion would not have been detectable by IA. This unique assay provides the ability to achieve an acceptable TAT, including the “stat” (4h) demands of a hospital emergency department to provide drug identification where more traditional methods typically require at least 24 hours.

P89

Bromazolam: Tracking Designer Benzodiazepines in Oral Fluid

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Introduction: Designer benzodiazepines are difficult to track in clinical laboratory settings where immunoassay-based screens and targeted confirmation analyses are routinely used. These difficulties can be mitigated by monitoring internal laboratory data trends, evaluating current regional and national drug trends, and routinely conducting cross-reactivity studies with laboratory immunoassay tests. Initial national bromazolam prevalence data commonly also reports that fentanyl is found in bromazolam positive samples. Though these processes will not catch all designer benzodiazepines, they promote clinical laboratories into proactive postures that allow them to identify more novel drugs and better serve their stakeholders.

Objectives: Our laboratory examined data from oral fluid samples from living individuals that screened positive for benzodiazepines by ELISA but confirmed negative for benzodiazepines by LC-MS/MS. Our objective was to re-test these oral fluid samples for bromazolam and assess its prevalence as an adulterant in this sample population.

Methods: Cross-reactivity tests were carried out by testing authentic oral fluid fortified with bromazolam reference material against our benzodiazepine ELISA plates (manufacturer targeted for 10 ng/mL oxazepam). This was done to ensure our authentic samples testing positive for benzodiazepines could be a result of bromazolam. We then evaluated all samples received by our laboratory after January 1st, 2024, using our laboratory management system to determine which samples screened positive for benzodiazepines by ELISA but confirmed negative for benzodiazepines by LC-MS/MS. This evaluation resulted in 400 unique oral fluid samples. Of these 400, we observed that 40 screened and confirmed positive for fentanyl.

Results: The cross-reactivity studies of bromazolam on the benzodiazepine ELISA plates showed that bromazolam had a cross reactivity of 333 %. Analyses of the preliminary data set consisting of samples that screened positive and confirmed negative for benzodiazepines (n = 400), revealed that 40 of those samples also screened and confirmed positive for fentanyl. These 40 samples showed a positive confirmation rate of 48.7% for bromazolam. Identified bromazolam positives (n = 40) were detected in Ohio (66.7%), Michigan (22.2%), Illinois (5.6%), and New York (5.6%) with concentrations ranging from 1.88 to 459.74 ng/mL. Additionally, of the confirmed bromazolam positives, 80% (n = 32) also confirmed positive for xylazine.

Discussion: Every identified sample that confirmed positive for bromazolam also confirmed positive for at least one other drug and in most cases several other drugs. Interestingly, most samples that confirmed positive for bromazolam and fentanyl also confirmed positive for xylazine, which supports the theory of bromazolam being used as an adulterant in illicit drug supplies. Coupled with the fact that we have detected bromazolam across four different states in the Midwest, this suggests that increased efforts of surveillance for designer benzodiazepines are warranted.

P90

Gabapentinoids Increasing in Montana

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Introduction: Gabapentinoids are a class of drug that consists of Gabapentin (Neurontin, Gralise, Horizant) and Pregabalin (Lyrica). These drugs are both anticonvulsants and used to treat neuropathic pain. They have been of rising concern in the forensic toxicological field in the United States due to a large increase in prescriptions (Young). As the Montana Forensic Science Division did not previously have a method to reliably detect gabapentinoids, an improved method to was developed. Since employing this method, the toxicology section has noted a significant increase in the number of gabapentinoid positive cases as well as the number of poly-drug cases involving gabapentinoids in conjunction with methamphetamine and amphetamine. Excluding ethanol, methamphetamine and amphetamine are seen with gabapentinoids at a higher rate than any other drug.

Objective: This study examined statistical data of cases positive for gabapentinoids and their combinations in poly-drug cases. The analytical method was developed specifically for gabapentinoids in whole blood using Shimadzu Micro Volume QuEChERS Kits (product number 225-37870-91) and LCMS/MS.

Methods: An internal standard mix was prepared with Gabapentin d10 and Pregabalin d6 in acetonitrile. Calibrators were prepared with blank blood and 1 mg/L certified reference standards from Cerilliant for a linear range of 0.5 to 50 mg/L. The extraction method was based on the application note by Shimadzu (Shimadzu). The QuEChERS tubes were prepared with HPLC water and acetonitrile before adding internal standard and 100uL of blood. The tubes were vortexed and centrifuged before transferring the supernatant for evaporation and reconstituted with 90:10 0.1% formic acid in water: 0.1% formic acid in methanol. Samples were analyzed on a Shimadzu 20AD XR LC system with an 8045 LCMS/MS. This method was validated based on ANSI/ASB Standard 036 and considered the following: selectivity, specificity, accuracy, precision, limits of detection and quantitation, carryover, dilution integrity, ion suppression/enhancement, and processed sample stability. Using this method, we collected data on the presence and concentration of gabapentinoids in whole blood for both postmortem and DUI cases.

Results: Since this method was employed in February of 2022, there has been a significant increase in the number of cases positive for gabapentinoids in Montana. Between 2021-2023, postmortem cases positive for gabapentinoids increased 78% and DUI cases increased 540%. In that timeframe, at least 28% of the cases positive for gabapentinoids were also positive for amphetamine or methamphetamine. Excluding ethanol, from 2021-2023, amphetamine and methamphetamine were the most common drugs detected with gabapentinoids in DUI cases, and the second and third, respectively, most common drugs detected with gabapentinoids in postmortem case in Montana.

Discussion: Gabapentinoid prescriptions are on the rise throughout the United States and globally. Between 2009 and 2016, gabapentinoid prescribing increased in every state ranging from 44%-176% (Pauly et al.). In 2020, the total number of gabapentinoid prescriptions was 80.11 million ("NFLIS-Drug Special Report: Gabapentin and Pregabalin Reported in NFLIS, 2011–2020"). However, even with the rise in gabapentinoid prescriptions, gabapentin is not federally controlled, though it is controlled in several states, while pregabalin is a Schedule V controlled substance (DEA Diversion Control Division). As scheduling for gabapentin is being discussed, both in Montana and nationally, proper detection and quantitation is integral.

Gabapentinoids are typically found with other medications or illicit drugs. So, while their presence in poly-drug cases is not surprising, the frequency of their use with methamphetamine in Montana is notable. Gabapentinoids are more commonly seen with fentanyl and heroin than methamphetamine in seized drug cases and are commonly prescribed with opioids, yet methamphetamine is seen more frequently with gabapentinoids in Montana ("NFLIS-Drug Special Report: Gabapentin and Pregabalin Reported in NFLIS, 2011–2020") (Peet et al.). With high prevalence of methamphetamine and amphetamine in Montana, it is yet to be determined if the combination with gabapentinoids is a deliberate choice of the user or incidental.

P91

Detection of Pharmaceutical and Illicit Drugs in Municipal Wastewater Using Gas Chromatography-Mass Spectrometry

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Introduction: Wastewater based epidemiology (WBE) is a field of study that utilizes wastewater to detect the presence of pharmaceutical and illicit drugs, pathogens, and chemicals within an area served by a wastewater reclamation facility (WWRF). Collecting samples from several WWRFs over the course of several weeks or months yields spatial and temporal datasets mirroring those reported by traditional survey methods. These traditional methods include population surveys, seizure records and hospital records. The traditional methods face issues with under-reporting, limited sample sizes, and the time needed for data collection and analysis. WBE has the advantage of providing faster, more accurate data for drug consumption within a community serviced by a WWRF.

Objectives: Apply a modified Gas Chromatograph-Mass Spectrometer (GC-MS) method for the detection of pharmaceutical and illicit drugs in wastewater in the greater Brimingham, Alabama area.

Method: Composite wastewater influent samples were collected at local WWRFs over a 24-hour period. The samples were filtered to remove large particles and were acidified to a pH of 2.5 for preservation. Solid-Phase Extraction (SPE) was used to extract the drugs of interest. Prior to this, a series of SPE cartridges were evaluated to determine the most suitable for the analyses. A comparison study between Pentafluoropropionic anhydride (PFPA) and N,O-Bis(trimethylsilyl) trifluoroacetamide (BSTFA) was conducted to determine the best derivatizing agent for the analytes Cocaine and Methamphetamine. Samples were derivatized and reconstituted in ethyl acetate, then analyzed using a GC-MS. A SIM and SCAN analysis with splitless injection was performed to monitor for selected compounds while retaining the ability to search for non-targeted analytes in the sample. Reference standards were purchased from Cerilliant (Round Rock, TX). MassHunter (Agilent, Santa Clara, CA) Unknown Analysis was used in conjunction with the NIST Library (v20, 2020), SWGDRUG library (3.13.L, 2023), Cayman library (v20022024, 2023) libraries to identify compounds of interest.

Results: SPE cartridges based on a broad spectrum copolymeric, cation exchange sorbent were found to be the most suitable for analysis. PFPA was suitable for most target and non-targeted analytes, but BSTFA was more suitable for benzoylecgonine, ibuprofen and gamma-hydroxybutyrate (GHB). The resulting qualitative data from the study identified acetaminophen, ibuprofen, cocaine, and methamphetamine in municipal wastewater through comparison with reference standards. A wider variety of substances were identified with MassHunter Unknowns Analysis, these include propofol, methaqualone, bezendrone, flumazenil, various synthetic cannabinoids, delta 8-THC, delta 10-THC, pregabalin, gabapentin, butonitazene, naproxen, methazolamide and LSD.

Discussion: This study, as far as the authors are aware, is the first qualitative data reported for the greater Birmingham area where selected drugs were monitored to determine the presence and the calculated amount of illicit substances consumed. Along with existing methods, WBE can provide a better picture of the extent of drug use for an area than traditional methods can alone. Qualitative data has the capability to identify substances that are new to an area and may be unaccounted for within existing datasets. The improved data will allow law enforcement and public health officials to take steps to prepare for the potential fallout from a new substance entering the community and adapt to changes in the drug(s) consumed within a community. This can lead to more effective strategies to prevent addiction, overdoses and drug-related violence. WBE has the potential to provide a reliable, real-time approach to tracking drug usage in a community.

The next course of study is to validate the WBE method for quantitative results and back-calculate to determine the extent of drug use within a community. Methamphetamine and cocaine have been selected for quantitation with the goal of determining average per capita drug use based on concentrations present in wastewater.

P92

Development and Validation of Liquid Chromatography Quadrupole/Time of Flight (LC/QTOF) Drug Screening Method for over 375 Drugs in Blood using Size Exclusion Chromatography (SEC) at ANSI/ASB Recommended Screening Thresholds

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Introduction: In the field of forensic toxicology, one of the most vital steps in any case examination is the drug screening process. Some of the most well-known and accessible screening methodologies contain analytical limitations. With the advancements in instrumentation, the field aims to pursue screening techniques with an expanded scope, enhanced sensitivity/specificity and reduced sample size while maintaining sample integrity. By using size exclusion chromatography (SEC), improvement in sensitivity for low-level analytes can be achieved by reducing biological matrix effects. The combination of SEC for sample preparation and LC/QTOF for analytical detection allows for the identification of analytes at recommended thresholds set by ANSI/ASB standards. This validation includes the identification of carboxy-THC and barbiturates by analyzing a single extracted aliquot in both a positive and negative method.

Objectives: To validate an LC/QTOF screening method utilizing SEC as a form of sample preparation at ANSI/ASB standard recommended thresholds.

Methods: Manual sample preparation is performed by a 500 µL aliquot of blood undergoes protein precipitation dropwise using 750 µL of cold acetonitrile. The sample is centrifuged for 10 minutes and transferred to a vial and dried to completion under nitrogen. Utilizing DPX®'s SEC Tips (1500 Da), the resin bed is rehydrated by placing the tip base into deionized water. Reconstitute the sample with 50 µL of 20:80 methanol: deionized water. The sample is vortexed and 40 µL is placed on to the hydrated resin bed to filter gravimetrically. The SEC tip is washed with 40 µL of 20:80 methanol: deionized and gravimetrically filtered through the tip to waste. The tip is transferred over a new vial to elute 300 µL of 50:50 methanol: deionized water and dried under nitrogen to completion. The sample is reconstituted in 100 µL of 95:5 mobile phase A (0.1% formic acid with 5 mM ammonium acetate in deionized water) and mobile phase B (0.1% formic acid with 5 mM ammonium acetate in methanol) to be analyzed on the instrument.

Analysis was conducted using an Auto MS/MS method on an Agilent 1260 Infinity II Liquid Chromatograph coupled with an Agilent 6546 QTOF. Chromatographic separation utilized a Phenomenex Kinetex 2.6 µm Phenyl Hexyl column (50 x 4.6 mm) paired with a phenyl guard column. Data analysis was performed using Agilent Mass Hunter Qualitative Analysis software in conjunction with our in-house Agilent Personal Compound Database Library (PCDL).

The following methods were implemented with a column flow rate of 0.500 mL/min.

Positive Method:

HPLC Gradient		
Time (min)	Pump A %	Pump B%
0.50	95.0	5.0
5.50	5.0	95.0
9.00	5.0	95.0
9.01	95.0	5.0
10.00	95.0	5.0

Negative Method:

HPLC Gradient		
Time (min)	Pump A %	Pump B%
0.0	60.0	40.0
2.0	2.0	98.0
3.0	2.0	98.0
3.6	60.0	40.0
5.1	60.0	40.0

Results/ Discussion: With the use of DPX®'s SEC tips, scope analytes are detected at the recommended thresholds. Utilizing one extraction method and two injections, this method successfully screens for 97% of 375 compounds within the validation scope. These compounds were detected at the lowest limits of detection across ANSI/ASB standards 119, 120, 121. Stability was determined to be 3 days post preparation for the samples. In the PCDL, there are 43 isomer groups of compounds that had potential for interference. Of those groupings, only 10 require combined entries for identification and will continue on for additional testing to confirm which isomer is present.

One limitation in the ability of LC/QTOF to eliminate traditional screening methods is the detection of carboxy-THC at the recommended threshold of 5.0 ng/mL. Reducing the matrix effects is crucial to the detection of carboxy-THC in the negative method at this concentration. With the utilization of DPX®'s SEC tips, improved signal to noise was attained for all analytes at the recommended limit of detection.

P93

Automated Workflows for Solid Phase Extraction of Fentanyl Analogs, Xylazine and Nitazenes in Urine and Oral Fluid

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Introduction: The opioid crisis has brought attention to the widespread abuse of fentanyl and fentanyl analogues, as well as nitazenes and xylazine. Fentanyl is a synthetic opioid used medically for pain management but has also been associated with many overdose-related deaths. Nitazenes are of the benzimidazole structural class and are synthetic opioids that were originally synthesized for use as an analgesic but are currently being abused for their opioid-like effects. Xylazine is a non-opioid sedative, analgesic and muscle relaxant, for sale in the United States only as a veterinary drug but has been reported as an adulterant in an increasing number of illicit drug mixtures.

The detection of these compounds in biological samples is crucial in clinical and forensic toxicology. Liquid chromatography-tandem mass spectrometry (LC-MS/MS) enables multiplexed detection of these compounds with high selectivity and sensitivity. However, achieving high-throughput with accurate results requires sophisticated sample preparation. This presentation demonstrates automated and semi-automated sample preparation for the extraction of these compounds from urine and oral fluid.

Objectives: Automating Solid Phase Extraction (SPE) and choosing optimized sorbent conditions offers advantages such as reduced error rate, increased walk-away time, and cleaner samples. This presentation demonstrates a comparison of analytical results from two SPE workflows: a semi-automated method utilizing the Tecan Resolvex® A200 and a fully automated workflow utilizing the Tecan Resolvex® i300 integrated on the Fluent® platform. Both Resolvex® instruments streamline SPE processing by automating sample loading, washing, and elution steps.

Methods: A reference set of fentanyl, fentanyl analogues, nitazenes and xylazine were utilized to demonstrate the automated SPE workflows. Using Utak drug free urine and the Quantisal saliva collection device to obtain 1mL of saliva, the biological matrices were then spiked with the compound standards resulting in a concentration of 1ng/mL. Tecan SPE columns were used to extract the analytes from human urine and oral fluid, using HPSCX and OFXQ SPE columns respectively. For each of the SPE workflows, both the Resolvex® A200 and i300 instruments were used for positive pressure loading and solvent dispensing, while the Resolvex® i300 also provided drying of the purified samples. Resuspended samples were analyzed using the Sciex 5500 QTrap and Sciex Zeno-TOF 7600 MS.

Results: The outlined workflow was evaluated analyzing efficiency, reproducibility, and overall robustness. LC-MS/MS analysis on the Sciex 5500 of the extracted samples provided ample sensitivity for the majority of the compounds, at a concentration of 1ng/mL in urine and oral fluid. To assess the efficiency of the extraction, the recovery of the samples was calculated using a post-extraction spike in. The SPE sorbent effectively extracted all fentanyl analogues, nitazenes and xylazines from human urine and oral fluid, yielding high recoveries across all tested compounds. 85% of samples analyzed showed recovery of 90% or greater. Technical replicate samples were processed to evaluate the reproducibility, demonstrating low %CV and high quantitative stability of the workflow. About two thirds of the samples analyzed resulted in %CVs lower than 10%. By utilizing the Tecan Resolvex® instruments, the SPE method was automated, resulting in a simple and efficient extraction protocol.

Discussion: The SPE methods have been proven to effectively extract a panel of 35 compounds consisting of fentanyl and its analogues, as well as nitazenes and xylazines from human urine and oral fluid. By utilizing the Tecan Resolvex® instruments, the SPE method was automated, resulting in a simple and efficient extraction protocol.

P94

The Analysis of Tianeptine in Whole Blood and Urine by Reverse-Phase SPE and LC-MS/MS

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Introduction: Tianeptine is an atypical tricyclic antidepressant not approved by the U.S. Food and Drug Administration (FDA) for medical use. It is being falsely marketed in gas stations and online as a dietary supplement under names such as “gas station heron”, “Zaza” and “Tianna Red”. At low doses, tianeptine helps regulate glutamatergic signaling making it an effective drug to treat depression and anxiety. At higher doses, tianeptine has an opioid-like effect because it is a full agonist at the mu-opioid receptor and a weak agonist at the delta-opioid receptor. There is a concern in the U.S. for misuse and there has been an increase in the number of tianeptine toxicity cases over the last few years. Due to the high doses of tianeptine being consumed, forensic laboratories need a procedure that has a high upper limit of quantitation. Additionally, tianeptine can be difficult to extract because it contains active acidic and basic groups. In a previous study, tianeptine recovery was low using a liquid-liquid extraction (LLE) method hypothesized because the compound is always ionized making it less likely to partition into the organic phase [1]. This poster outlines an optimized solid phase extraction (SPE) procedure utilizing UCT’s Styre Screen® HLB for the extraction of tianeptine from blood and urine with high recoveries.

Objectives: Develop analytical and extraction methods for the analysis of tianeptine with a linear range relevant to concentrations a forensic or medical examiner laboratory may encounter. Determine what mechanism of solid phase extraction results in the highest recovery.

Methods: Previous to the reverse phase method, ion exchange SPE using UCT’s Clean Screen DAU SPE column was trialed. Recovery from these trials averaged about 60%. Higher recovery was achieved with the following method. Samples were extracted using UCT’s Styre Screen® HLB 60 mg, 3 mL SPE column. 200 µL of sample was diluted with 2 mL of 100 mM phosphate buffer (pH = 6) before being vortexed and centrifuged. SPE columns were conditioned with 3 mL of methanol and 3 mL of 100 mM phosphate buffer (pH = 6). After loading the sample, SPE columns were washed with 3 mL of 100 mM phosphate buffer (pH = 6) and 3 mL of 10% MeOH in DI H_2O. SPE columns were dried under full pressure for at least ten minutes before eluting the analyte with 3 mL of DCM:IPA:NH4OH (78:20:2). Samples were evaporated and reconstituted with 1 mL of MeOH:H_2O (50:50).

Samples were analyzed on a Shimadzu Nexera LC-30AD with MS-8050 equipped with a SelectraCore® C18 LC Column (50 x 2.1 mm, 2.7 µm) and SelectraCore® C18 guard column (5 x 2.1 mm, 2.7 µm).

Results: Recovery, matrix effect, and relative standard deviation at two concentrations, 25 ng/mL and 750 ng/mL, in blood and urine (n=5) were calculated to assess the extraction procedure. Recovery for all samples ranged between 87% to 96%. While matrix effects ranged between ± 25% with no significant impact on sample quantitation (-19% to 24%). Lastly, the relative standard deviation for all samples was less than 10%.

Discussion: A method was developed with a linear range of 20 ng/mL to 1000 ng/mL only using 200 µL of sample. The large linear range will allow laboratories to analyze potential tianeptine toxicity cases limiting the number of samples that will have to be repeated with dilution. Using less sample and reconstituting with a higher volume prevents the mass spectrometer from being overloaded. Utilizing UCT’s Styre Screen HLB and a reverse phase SPE mechanism the method achieved high recoveries. It’s presumed that an ion exchange SPE mechanism was unsuccessful due to tianeptine’s amphoteric nature.

P95

Detection and Quantification of Carbon Monoxide in Postmortem Liver Tissues via Head-space GC-MS as an Alternative to Blood

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Introduction: Carbon monoxide (CO) is a gas that is readily absorbed by the lungs and into the blood stream when inhaled. Inhalation can occur during incidents such as attempted suicides and fires, and can result in toxic and lethal effects when inhaled in high amounts. Once in the blood, CO forms a complex with hemoglobin (Hb) in red blood cells (RBC) known as carboxyhemoglobin (COHb), displacing the oxygen-carrying capacity of Hb and therefore disrupting the transport and delivery of oxygen throughout the body, ultimately resulting in death due to lack of oxygen. Because of the known lethal potential of CO, it is important to be able to detect it in postmortem samples for relevant cases when determining a cause of death. Blood and spleen are the prevalent sample sources for the detection of CO because they are Hb rich sources, but these samples may not always be available for collection during autopsies.

Objectives: The aim of this study is to determine if liver can be a reliable source for the detection and/or quantification of carbon monoxide in postmortem cases in the absence of an available blood or spleen sample.

Methods: Blood and liver homogenate samples from 25 postmortem cases known to be positive for CO (%COHb >10%) and 10 postmortem cases known to be negative for CO (%COHb <10%) were analyzed for carboxyhemoglobin via head-space gas chromatography/ mass spectrometry (HS-GC/MS). Two aliquots of each specimen are prepared, one of which is saturated with CO. The CO is liberated from the hemoglobin and analyzed by HS-GC/MS. The %COHb saturation of the sample is determined by the ratio of the CO peak area in the unsaturated specimen to the CO peak area of the saturated specimen. Blood and liver %COHb results for the same cases were compared.

Results: Liver results did not correlate well with blood results. Cases in which the blood produced a positive result for CO did not always result in a positive finding in liver, even in cases with high levels of %COHb saturation (>50%). Cases that did produce a positive result in liver showed variability between the corresponding quantitative results in blood and liver. However, all cases that had a liver result ≥10% COHb had a corresponding blood result ≥10% COHb.

Discussion: Liver tissues may not be as hemoglobin rich as blood and spleen, and therefore are not a reliable source for quantification of %COHb. It should also be noted that liver tissues contain additional iron(II) binding proteins such as cytochromes which may provide additional binding sites for CO during the saturation step of sample prep, potentially skewing %COHb results in liver tissue to be lower than they truly are.

If blood or spleen is not available to be tested for the detection of CO, liver may be tested as a potential indicator of CO intoxication. A positive %COHb finding in liver correlates with a positive %COHb result in blood, but not in such a way that a quantitative blood result can be extrapolated from a liver result. Negative results in liver are inconclusive and cannot rule out CO poisoning.

P96

Analysis of Synthetic Cathinones From Blood and Urine Using Clean Screen® Xcel I on LC-MS/MS

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Introduction: Synthetic cathinones also known as 'bath salts' are a group of novel psychoactive substances (NPS) with stimulant effects that are structurally related to the naturally occurring alkaloid cathinone. These stimulants cause an increase in the activity of the central nervous system (CNS). Like other groups of NPS, forensic laboratories work hard to update their scope of analysis to keep up with the ever-changing market. The newest popular synthetic cathinones are N,N-dimethylpentylone and α-PHP. These basic compounds can be easily implemented into extraction and analytical methods with other common stimulants such as methamphetamine, amphetamine, and MDMA. UCT's Clean Screen® Xcel I is a proven top-tier SPE column that can extract a wide array of basic compounds. In conjunction with UCT's SelectraCore® DA (biphenyl) core-shell LC column, a method for analyzing synthetic cathinones and other common stimulants from blood and urine was successfully developed.

Objectives: The purpose of this project was to develop analytical and extraction methods for the analysis of synthetic cathinones from blood and urine. The analytical method needed to include the separation of isomers eutylone and butylone.

Methods: Blood and urine samples were extracted using UCT's Clean Screen® Xcel I SPE columns. 0.5 mL of sample (blood or urine) was diluted with 1.5 mL of 100 mM phosphate buffer pH 6 before being vortexed and centrifuged. Due to the proprietary formula of Xcel I columns, samples can be applied to SPE columns without conditioning. After loading samples, SPE columns were washed with 3 mL of 1 M acetic acid (aq.) and 3 mL of methanol. The sorbent was dried under full pressure for at least 10 minutes before eluting with 3 mL of EtOAc:IPA:NH_4OH (80:18:2). 100 µL of 1% HCl in methanol was added to the elute to prevent analyte loss during evaporation. After evaporating samples to dryness at 5 psi, 35°C, they were reconstituted with 0.5 mL of 95:5 (H_2O: MeOH).

Samples were analyzed on a Shimadzu Nexera LC-30AD with MS-8050 equipped with a SelectraCore® DA Column (50 x 2.1 mm, 2.7 µm) and guard column (5 x 2.1 mm, 2.7 µm). The LC gradient was a simple linear progression from 5% B to 100% B over 8 minutes. The whole method was 13.20 minutes long.

Results: The SPE method was evaluated by calculating the recoveries, matrix effects, and relative standard deviations for a low and high concentration (25 ng/mL and 200 ng/mL). Urine samples (n=5) had recoveries ranging between 97% and 106%. Matrix effects were low ranging between -7% and 1%. The relative standard deviation was less than 10%, ranging from 2% to 7%.

Blood samples (n=5) had recoveries ranging between 80% and 102% and relative standard deviations ranging between 5% and 17%. Blood samples were subjected to more ion suppression than urine samples, but the limit of detection and quantitation were not affected. Matrix effect ranged between -32% and -5%. The method had a limit of detection of 5 ng/mL.

Discussion: This poster outlines a method for the simultaneous analysis of synthetic cathinones and other commonly encountered stimulants. The SelectraCore® DA (biphenyl) column was selected for its ability to separate isomers eutylone and butylone. The utilization of Clean Screen® Xcel I helps reduce solvent usage and extraction time by allowing users to bypass the conditioning and equilibration steps of SPE. The extraction and analytical methods were successfully evaluated using pre and post-spiked blood and urine samples to calculate recoveries, matrix effects, and relative standard deviations.

P97

Determination of the Aerosolization Efficiency of Nicotine and Ethanol in a Eutectic Mixture in E-liquids

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Introduction: Electronic cigarette (e-cig) liquids (e-liquids) are typically formulated as a mixture of propylene glycol (PG) and vegetable glycerin (VG), flavorants, and pharmacologically active compound such as nicotine. Technological advancements have enabled e-cigs to be co-opted for use with drugs other than nicotine (DOTNs), such as ethanol, herbal products, or illicit substances. The interaction between substances can form a eutectic mixture, which reduces the melting temperature to a point lower than the temperature of the individual compounds, promoting aerosolization. Previous studies have demonstrated that e-liquids with equal parts nicotine and methadone resulted in increased aerosolization and recovery of both substances, as compared to respective single drug e-liquids at the same concentration.

Objectives: The purpose of this experiment was to determine the effect of ethanol on the aerosolization and recovery of nicotine generated by an e-liquid in an e-cig using an in-house aerosol trapping system.

Methods: E-liquids were created in-house using PG to VG ratios of 100:0, 70:30, 50:50. 30:70, and 0:100 with 12 mg/mL nicotine, and ethanol concentrations of 0, 2, 5, 10, 15, 20, and 25% ethanol in each PG:VG matrix. Aerosol capture was performed on 35 e-liquids (n=5) using a flask trap system with a Kanger SUBOX Mini e-cigarette device (resistance (Ω=0.65), power (W=28.4) and voltage (V=4.3)). Each trial consisted of 10 puffs for a duration of 4 seconds. The device tank was weighed before and after each puffing session. Samples were analyzed by Headspace-Gas Chromatography-Flame Ionization Detection and Liquid Chromatograph- Tandem Mass Spectrometry to determine the concentration, dosage, and percent recovery of ethanol and nicotine, respectively.

Results: The volume of e-liquids aerosolized in 100% PG was 572.1 µL (CV=8%) and 639.5 µL (CV=4%) for 0% and 25% ethanol, respectively. The volume of e-liquids aerosolized in 100% VG was 450.5 µL (CV=11%) and 469.5 µL (CV=8%) for 0% and 25% ethanol, respectively. The recovery of nicotine in e-liquids with 0% ethanol was 69% (CV=26%) and increased to 85% (CV=7%) in e-liquids with 25% ethanol concentrations in 100% PG. The recovery of nicotine in e-liquids with 0% ethanol was 81.5% (CV=11%) and increased to 85.0% (CV=6%) in e-liquids with 25% ethanol concentrations in 100% VG. Ethanol recoveries decreased from 229.3% to 148.3% in e-liquids with ethanol concentrations of 2% to 25% in 100% PG and 160.4% to 139.8% in e-liquids with ethanol concentrations of 2% to 25% in 100% VG.

Discussion/Conclusion: Increasing concentrations of ethanol in e-liquids decreased viscosity and increased aerosolization, with linear increases of nicotine dose. E-liquids comprised of 100% PG have a lower density compared to 100% VG, that inherently lead to greater volatilization, further aided by the addition of ethanol. In contrast, e-liquids in 100% VG have higher densities and viscosity, keeping the volume aerosolized more consistent despite increasing ethanol concentrations. The matrix composition was found to change the liquid aerosolization and recovered dose of nicotine Increased drug availability from the unintended formation of eutectic mixtures can lead to untoward effects. While experienced consumers self-titrate the amount of drug consumed to manage effects, the increased dose delivered can facilitate substance addiction.

P98

If You Can't Be With the Ethanol You Love, Love the Methanol You're With: A Look at Methanol Poisoning Cases in Miami-Dade County

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Introduction: Methanol and ethanol are both alcohols; however, methanol metabolism to formic acid makes it extremely toxic, producing anion gap metabolic acidosis, blindness, organ failure, encephalopathy, coma, and death. Despite its toxicity, a long history of accidental methanol consumption exists, with poisonings reported since the late 1800s, typically from ingestion of home-distilled alcohol. During alcohol fermentation, methanol is formed as a byproduct and individuals are exposed when drinking alcohol in which the methanol component has not been removed. During Prohibition, methanol was intentionally added to alcoholic beverages in an effort to prevent consumption. This effort failed; consequently, poisonings were common. Still today, most methanol poisonings are due to intentional or accidental ingestions. Methanol is found in many common industrial and household products, making it an easy substitute for ethanol. Occasionally still, fermenting and distilling errors cause beverage contamination leading to accidental poisonings as well. However, cases are almost exclusively isolated incidents with occasional epidemic outbreaks.

Objectives: The presentation objectives are to offer an overview of methanol deaths in Miami-Dade County from 2010 to present, to characterize the seven deaths at the Miami-Dade Medical Examiner Department (MDME) in which methanol detection was not attributed to embalming fluid, and to highlight one of those cases.

Methods: A query was conducted for MDME methanol positive cases from 2010 to present. Cases in which methanol detection was attributed to embalming fluid (92%) were excluded and the remaining cases (8%) were characterized, including demographics, manners and causes of death, and concentrations. Methanol is detected and quantified in routine toxicology casework utilizing HS-GC-FID technology.

Results: From 2010 through May 2024, 88 cases were received in which methanol was identified in toxicology testing. The vast majority (92%) were funeral home cases under MDME jurisdiction that were already embalmed. Embalming fluid explains methanol identification in these cases. Only 7 decedents (8%) were not embalmed. There were two natural deaths, involving heart disease and human immunodeficiency virus, and one undetermined manner of death involving bronchopneumonia. There were three accidental deaths, one with the cause of death blunt force trauma. The other two accidental cases, along with one suicide, were attributed to acute methanol toxicity. Demographically, most decedents were white males (71%) and their ages ranged from 26 to 71 years. Blood concentrations ranged from detected less than the limit of quantitation (0.02%) to 0.225%.

One highlighted case involves a 40-year-old white female who died in the hospital. Testing was negative for ethanol; however, a reference lab identified methanol at 0.013% in a source unknown to the MDME. Although the husband's ever-changing story led the decedent's parents to believe he had poisoned her with methanol, the investigation revealed that the decedent abused ethanol and when ethanol was unavailable, she would ingest other substances. No suspicious beverages were found at the scene and no evidence of foul play was discovered. Toxicology revealed a negative result for ethylene glycol; however, methanol was quantified at 0.184% in antemortem blood and 0.196% in antemortem serum. The large concentration discrepancy with the reference lab could be attributed to ongoing methanol absorption into the bloodstream and the MDME testing a later obtained specimen. The cause of death was reported as acute methanol toxicity. Only the manner of death was in question and based on the investigation, autopsy and toxicology findings, as well as lack of evidence of foul play, the medical examiner ruled the case to be accidental.

Discussion: Methanol detection in Miami-Dade County is almost exclusively due to embalming fluid. Infrequently, an isolated accidental or intentional poisoning occurs. Methanol is an age-old poison that tends to rear its ugly head from time to time.

P99

Carfentanil Goes West

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Introduction: Carfentanil is an opioid historically used to sedate large animals such as elephants with a potency approximately 10,000 times greater than morphine. Due to its high potency, carfentanil can be difficult to detect in toxicology casework with impairing and fatal concentrations starting as low as 0.02 ng/mL. Carfentanil started appearing in Toxicology casework nationwide in 2016. However, it had not been regularly detected in Orange County, CA in neither Toxicology nor Seized Drug cases until late 2023.

Objectives: To evaluate trends in carfentanil detection in Orange County, CA, and to determine concentrations of carfentanil in current antemortem and postmortem (PM) blood and tissue samples. Cases positive for carfentanil were reviewed to determine the frequency of detection and concentrations were determined in blood samples. A quantitative method was developed for blood and tissue samples to evaluate concentrations. Case studies will be presented.

Methods: Blood samples determined positive for carfentanil (LOD 0.2 ng/mL) by a liquid chromatogram-quadrupole time of flight (LC-QTOF) screen were quantified along with any other biological samples collected for the cases. A sample size of 0.5 mL of blood, urine or vitreous humor or 0.5 g for tissue was mixed with 0.1 mL of deuterated internal standard (carfentanil-13C6) then extracted by protein precipitation using 1.5 mL cold acetonitrile and centrifugation. The supernatant was aspirated using DPX® WAX tips to further remove impurities in the sample and then 0.2 mL was transferred to a vial with 0.90 mL of water. Quantitative analysis was performed by a Waters Aquity liquid chromatograph (LC) -tandem mass spectrometer with a XeVo TQ-S utilized in ESI+ mode. The LC used a Phenomenex Kinetex Biphenyl column (2.1 x 100 mm) with aqueous and organic phases of 100% water and acetonitrile, each with 0.1% formic acid and a run time of 6 minutes. This method was validated following ASB/ANSI 036 validation standards. The quantitative range was 0.05-10 ng/mL on a quadratic curve, weighted 1/x and was for used research purposes only.

Results/Discussion: Beginning in November 2023, 28 cases either screened positive or had an indication of possible carfentanil; 12 were human performance bloods and 16 were death investigation cases. Carfentanil blood concentration ranged from <0.05 ng/mL to 5 ng/mL. Higher concentrations were observed in the liver, brain and vitreous humor while none was detected in the gastric contents. All 28 cases also had fentanyl present, ranging from 0.6 – 100 ng/mL in the blood. Methamphetamine was the second most common drug detected with carfentanil with 22 (78%) of the cases. This is similar to the submissions the Seized Drugs section has reported with low amounts of carfentanil being present with fentanyl. The Seized Drugs submissions containing carfentanil were described as beige, light brown, and/or white substances, very similar to the majority of their fentanyl submissions.

Carfentanil use has progressed west since 2016. In many states, carfentanil was attributed to thousands of overdoses. However, in Orange County, carfentanil has often been seen mixed with other drugs in both antemortem and postmortem samples, leading to the possibility of carfentanil being used as a cutting agent or contaminant, rather than the sole drug being distributed.

P100

The Heat Is On - Pushing Detection Limits of LC-QTOF-MS Screening with a VIP-HESI Source

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Introduction: The use of liquid chromatography time-of-flight mass spectrometry (LC-TOF-MS) for screening of bodyfluids has increased significantly in recent years. Non-targeted acquisition of high resolution (HR) MS data allows for the detection of a theoretically unlimited number of compounds, retrospective data analysis, and possible identification of unknowns. All features that are impossible with other MS methods. However, these instruments usually lack the sensitivity of a high-end triple quadrupole MS which might become an issue, especially when dealing with low concentration drugs or drug facilitated crimes. The most obvious way to improve sensitivity is to increase the ion yield of the source. Vacuum insulated probe heated electrospray ionization (VIP-HESI) shows a significant gain in signal intensities when compared to standard electrospray ionization (ESI) and will therefore likely lead to lower limits of detection (LODs) when analyzing bodyfluids.

Objectives: Optimization of the source parameters of a VIP-HESI source to lower detection limits of a routine LC-ESI-QTOF screening (TargetScreener HR, Bruker Daltonik).

Methods: A set of 120 most frequently detected substances in our lab in the last 24 months was selected for parameter optimization and subsequent evaluation. Further compounds of special interest, e.g. synthetic cannabinoids and nitazenes, were added. Source parameters having the greatest influence on the ionization efficiency, like capillary voltage, dry gas and probe gas parameters were optimized using standard mixtures and spiked pooled human urine (n = 10). To assess the effects, the amount of an n-dimensional vector, including peak areas of all compounds investigated, was calculated for each parameter setting.

To determine LODs with the final settings, 10 blank human urine samples were spiked in descending concentrations starting at 50 ng/mL. 100 µL aliquots were extracted using cold acetonitrile and reconstituted in 25 µL of LC eluent. The analysis was performed using 20-minute gradient elution on an Elute UHPLC connected to an impact II TOF (TargetScreener HR, Bruker Daltonik) equipped with a VIP-HESI source. Data evaluation was performed automatically using the routine TASQ® method.

Results: VIP-HESI standard parameters already led to an increase of peak areas by a factor of two to nine. This could be further increased by optimizing the source parameters, e.g. for a set of 12 nitazenes, peak areas increased by a factor of nine to eleven. In general, high capillary voltage, high probe gas temperature, low nebulizer pressure and low dry gas flow were most beneficial.

Due to the low noise levels when using HR-MS, the signal gain observed in eluent was also clearly visible in matrix. Compounds at the upper end of the LOD range (c = 50 ng/mL) of our routine approach, e.g. 6-monoactylmorphine, norfentanyl, norbuprenorphine or methamphetamine, could now be easily detected at 10 or 5 ng/mL, respectively. LODs of some compounds, e.g. alprazolam or sufentanil, fell below the previous value of 1 ng/mL. No negative effects for matrix samples could be observed.

Discussion: Implementation of a VIP-HESI source led to significant increase in signal intensities, compared to standard ESI. In general, the signal-to-noise ratio is considered a better parameter to judge the sensitivity of a method than signal intensity. However, due to the very low noise of HR data, the peak area was chosen as criterion during method development. Optimization of the VIP-HESI source parameters, mainly capillary voltage and probe gas settings, led to further enhancements for the majority of compounds. Compared to the validated method using ESI, this led to LODs down to 1.0 ng/mL and below in urine. This enables a prolonged detection window or reduction of sample volume.

P101

Cannabinoid Cross-Reactivity Using Four Oral Fluid Instant Test Devices

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Introduction: In the United States, marijuana is a federally illicit substance. Due to changes in state laws marijuana is now legal for purchase in certain states. The change in laws has increased access to and the amount of people who are using this drug. As such, certain stakeholders in public health and safety—like child protective services and probation departments—require fast and effective testing methods to detect Delta-9-THC. In this study, four oral fluid instant tests were evaluated for detecting Delta-9-THC and other cannabinoids to determine their cross-reactivity and accuracy relative to a laboratory test.

Objectives: The purpose of this experiment was to evaluate if oral fluid instant tests are accurate and appropriate for use in point-of-care settings compared to laboratory tests.

Materials and Methods:

The four multi-panel oral fluid instant tests evaluated were DrugWipe 6S, StatSwab, Orawell, and OralTox. These tests collect oral fluid samples and bind with an antibody coating to display a positive or negative result. Each was evaluated for a series of cannabinoids based on the instant device Delta-9-THC cutoff concentrations. Delta-8, Delta-9, and Delta-10-THC, and a cannabinoid mixture (CBD, CBDA, THCA, THCV, CBG, CBDV, Cannabinol, and CBC) solutions were created at 100X, 10X, 2X, Cutoff (1X), and 0.5X concentrations. A negative control (synthetic negative saliva) was also tested. Not all the instant tests had cutoffs listed for these analytes, so the Delta-9-THC cutoff concentration was used as the basis for these solution concentrations. Each analyte/mixture were tested at each concentration in triplicate.

Results: Data was compiled for each analyte/mixture at each concentration for each instant test. If samples yielded a negative result in the Cutoff (1X), 2X, 10X, or 100X cutoff concentrations, those tests were marked as false negatives. Additionally, tests that were positive for a concentration below the Cutoff (1X) were labeled as a false positive. Samples that were positive at the Cutoff (1X) and above were labeled as true positives; tests that were negative below the Cutoff (1X) were classified as true negatives. The compiled data across all 96 instant tests showed 66% false negative, 8% false positive and only 26% of the tests displaying an accurate result.

Discussion: There was variation between the instant tests and their ability to detect Delta-9-THC and other common cannabinoids. The data suggests that these instant tests were mainly designed to detect Delta-9-THC, compared to Delta-8, Delta-10, and the cannabinoid mixture components. Drug Wipe 6s and OralTox instant tests demonstrated poor accuracy for Delta-9-THC (<56%). Orawell could detect Delta-8, Delta-9, and Delta-10-THC with 100% accuracy and the cannabinoid mixture with 83% accuracy. In contrast, OralTox was only able to detect Delta-8, Delta-9, and Delta-10-THC, and the cannabinoid mixture with 55.6% accuracy. The instant tests varied in their performance detecting Delta-9-THC and their cross-reactivity with different cannabinoids. These devices can certainly serve as a tool for identifying cannabinoids, but it is always recommended to have a confirmatory laboratory test to identify the true cannabinoid(s) being measured.

P102

An Analysis of Drug Detections in Carfentanil Cases Across the Country from 2020-2024

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Introduction: Carfentanil is a fentanyl derivative that emerged in the illicit drug market in 2016-2017 and exhibits much greater potency than morphine and fentanyl. A non-human tail withdrawal test estimates the potency of carfentanil to be 100x fentanyl and 10,000x morphine. Similar to fentanyl and its other analogs, carfentanil's primary mechanism of action is at the mu opioid receptor. In paper published in 2017, 7 cases from 2017 were reported to have markers of heroin detected, in addition to carfentanil. 6 out of 13 cases from 2016-2017 cited at least one additional drug besides carfentanil in the cause of death statement. Single drug overdose deaths have fallen in recent years. Additionally, fentanyl has mostly replaced heroin as the primary opioid detected in street drugs and is often found with other NPS opioids in casework. It can found in polydrug overdose death cases involving carfentanil.

Objectives: We examined the data from our lab from January 2020 to May 2024 to see what drugs were detected in blood with carfentanil. 82 compounds that are considered novel psychoactive substances (NPS), fentanyl, methamphetamine, benzoylecgonine, morphine, delta-9-carboxy-THC, and xylazine were considered in the scope of this project.

Methods: Our lab receives postmortem blood specimens in sodium fluoride preservative tubes. Carfentanil is screened in our comprehensive panel using a cold acetonitrile extraction and injection on a liquid chromatography quadrupole time of flight mass spectrometry (LC-QToF-MS) system. The confirmation test is performed using a protein precipitation extraction and then injection on a liquid chromatography triple quadrupole mass spectrometry (LC-MS/MS) system. The lower limit of quantitation (LLOQ) in blood is 20 pg/mL. The limit of detection (LOD) is 10 pg/mL with specimens reported qualitatively positive between 10 and 20 pg/mL.

Results: During this time period, there were 219 detections of carfentanil in blood samples. The most common of the NPS compounds included acetylfentanyl (n=92), fluorofentanyl (n=22), and bromazolam (n=6). 4 nitazene compounds were detected which were metonitazene (n=2), metodesnitazene (n=1), N-pyrrolidino metonitazene (n=1), and N-pyrrolidino protonitazene (n=1). Other NPS compounds detected were N,N-dimethylpentylone, and butyrylfentanyl with 2 detections each, and etizolam, pentylone, alpha-PiHP, brorphine, acrylfentanyl, cis-3-methylfentanyl, cyclopropylfentanyl, and methoxyaceylfentanyl with 1 detection each. Detections for the common drugs of abuse examined included fentanyl (n=152), methamphetamine (n=63), delta-9-carboxy-THC (n=46), benzoylecgonine (n=40), morphine (n=33), and xylazine (n=5).

Discussion: 2021 and 2022 saw 9 and 2 detections in casework respectively which is a drop off from 109 in 2020. In June 2023, we noticed a cluster of several cases with carfentanil which is when we decided to monitor the trend. The second half of 2023 saw 46 detections and January to April 2024 saw 61 detections. 30 out of 219 cases with carfentanil detected had no other drugs of interest present. Further examination of those cases showed that 20 of the cases were only tested for carfentanil and did not include any kind of screen testing. The remaining 10 cases were screened for other drugs and none of note were detected. 11 cases with carfentanil and methamphetamine detections did not have fentanyl detected. 11 cases with carfentanil and benzoylecgonine detections did not have fentanyl detected and 4 of those cases had parent cocaine. N-pyrrolidino metonitazene and N-pyrrolidino protonitazene were detected in the same case with fentanyl present. The 5 cases with xylazine all had fentanyl detected.

P103

Bromazolam and Fentanyl in Postmortem Blood Samples

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Introduction: Bromazolam is a novel benzodiazepine, which was originally developed for therapeutic use in 1976, but has recently become prevalent in the illicit drug supply. Bromazolam has been observed to be found often in conjunction with fentanyl in postmortem blood samples. Seized drug reporting supports this, as this combination has been confirmed in purported "benzo-dope" exhibits, which are combinations of illicit opioids and benzodiazepines. The contaminated drug supply poses increased risk to end users, due to the potential for enhanced effects. The toxicology data coupled with drug checking efforts implies the co-detection of both bromazolam and fentanyl is likely due to the use of one product containing multiple substances, and not necessarily individual exposure to each drug.

Objective: The aim of this study is to evaluate the prevalence of both bromazolam and fentanyl in postmortem cases.

Methods: Results for postmortem blood specimens received and reported between 2020 and 2023 and screened for fentanyl and bromazolam were extracted from the laboratory information management system (LIMS). Comprehensive screening of casework was performed using liquid chromatography time of flight mass spectrometry (LC-TOF/MS). Fentanyl was included in the scope of comprehensive screening, with confirmatory testing automatically triggered after a positive screen. Bromazolam was monitored through the LC-TOF/MS surveillance library, which ran concurrently with the published scope of expanded testing. However, confirmatory testing for bromazolam was only performed after approval or original request from the submitting agency. Quantitative confirmation for both fentanyl and bromazolam was achieved using liquid chromatography tandem mass spectrometry (LC-MS/MS).

Results: Bromazolam emerged in 2020 with one positive case reported, but positivity started to increase in 2021, 2022, and 2023 with 123, 730, and 1936 reported cases respectively. A population of 2790 postmortem blood samples all positive for bromazolam, were used for analysis of co-reporting, with samples being analyzed from 39 states and 5 Canadian provinces. The gender breakdown was 68% male, 25% female, and 7% unknown. Of this sample set, 86% of samples reported both bromazolam and fentanyl, with 433 samples screening none detected for fentanyl. Bromazolam blood concentrations ranged from 2 to 5900 ng/mL, while fentanyl blood concentrations ranged from 0.27 to 13000 ng/mL. The average bromazolam concentration was 57 ng/mL, with a median concentration of 22 ng/mL and average fentanyl concentration was determined as 42 ng/mL, with a median of 11 ng/mL. For postmortem cases with low concentrations of bromazolam, between 2.0- 5.0 ng/mL, fentanyl was reported in 86% of those cases. Comparatively, in bromazolam cases reported at greater than 1000 ng/mL, fentanyl was reported in 50% of those cases.

Discussion: Postmortem samples rarely contain bromazolam without fentanyl. Increased reporting of bromazolam has been observed, but this could be attributed to the availability of a routine confirmatory test in May 2023. Further, the co-reporting of bromazolam and fentanyl in postmortem samples does not appear to be geographically isolated; cases in this population were reported near bodies of water, including both coasts of the United States and around the great lakes. Although it is possible that the high percentage of co-reporting is due to individual prevalence, seized drug reporting of "benzo-dope" corroborates these toxicology findings. Bromazolam and fentanyl when ingested together can have an additive effect on the central nervous system (CNS). The combined effects can lead to increased depression of the CNS resulting in an increased mortality rate. It is possible that many users may not know they are being exposed to bromazolam, especially if users are intending to purchase illicit opioids. Bromazolam has been proved to be incorporated in the illicit drug supply, and can be used by itself or in combination with other drugs. It is also probable that bromazolam is underreported due to lack of inclusion in most routine toxicology tests.

P104

GC-MS Analysis of Tetrahydrozoline in Alcoholic Beverage Residue Related to Drug-Facilitated Sexual Assault

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Introduction: Drug-facilitated sexual assault (DFSA) is an ongoing issue that continues to plague not just the nation but the globe as well. Drugs commonly found in DFSA cases in the United States include ethanol and cannabis in combination with other drugs. However, alternative drugs are increasingly being used to incapacitate victims to enable a sexual assault, such as tetrahydrozoline (THZ). THZ is commonly found in over-the-counter eye drops, such as Visine®. With minimal research surrounding THZ in DFSA cases, agencies and institutions may process incidents with a general drug screen. The general screen may only detect common drugs, with THZ going undetected. Additionally, alternative samples for analysis are of interest in cases where biological samples cannot be used due to the metabolism and excretion of the drug before the sample is collected. Therefore, investigations of new drugs in DFSA should be accompanied by analyses of alternative sample types, such as beverage residues in containers, to aid in situations where the drug cannot be identified in a biological sample received for testing.

Objectives: The research objectives were to develop a method to detect tetrahydrozoline in alcoholic beverage residue using Gas Chromatography-Mass Spectrometry and assess the detectability of THZ residue spiked in an alcoholic beverage in different cup materials.

Methods: Ten milliliters of High Noon Hard Seltzer Vodka Lemon, 4.5% by volume, was poured into red solo cups, clear chinet plastic cups, Styrofoam cups, and clear drinking glasses, then spiked with 100 µg/ml THZ. The beverage was poured out, and the containers dried overnight. The dried residue was redissolved in methanol, extracted using liquid-liquid extraction, and the extract evaporated. Samples were reconstituted and analyzed using GC-MS at a 1:15 split ratio. The same steps were repeated with the High Noon spiked with different volumes of Visine® eye drops. All analyses were conducted in triplicate. The validation parameters assessed using the THZ standard were repeatability, limit of detection (LOD), autosampler stability, and linearity. For autosampler stability, the THZ standard was analyzed pre-extraction.

Results: THZ (from the reference standard) and THZ (from Visine®) were detected in the residue of each cup type. However, recovery of THZ was more efficient from the chinet and red solo cups as assessed by peak area ratio. Repeatability studies yielded relative standard deviations (RSD) below 13% at both lower (20 µg/ml) and higher concentrations (100 µg/ml). A calibration curve analysis determined the LOD of THZ to be 20 µg/mL. THZ was stable for 9 hours under the autosampler stability study based on the graph of the peak area ratios (PAR) versus injection time, having a positive slope that was not statistically significant from zero. The THZ residue from the various containers was quantitated and determined to be below the method's LOD. However, preliminary findings indicate that the chinet and red solo cups yielded higher concentrations of the residue, supporting the recovery study.

Discussion: Under the conditions of the study, THZ was detectable in residue from a spiked alcoholic beverage. The calculated PARs and RSDs conveyed the method was repeatable. The findings of this study have significant practical implications. They allow the use of alternative samples in DFSA cases when biological samples are unavailable, thereby enhancing the efficiency of forensic investigations. Further research includes comparing solid phase extraction and liquid phase extraction for THZ, exploring derivatization of THZ with pentafluoropropionic anhydride, increasing the volume of the alcoholic beverage and lowering the concentration of THZ spiked, and using splitless injection. These measures will allow for a lower LOD and limit of quantitation (LOQ), increasing the method's sensitivity. Additionally, the steps mentioned for further research will enable quantitating findings to determine the detectable concentrations of THZ in Visine associated with the number of drops used.

P105

An Automated Dispersive Pipette Extraction for Sensitive Analysis of Alcohol Metabolites in Common Forensic Matrices

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Introduction: Various extraction methods for alcohol metabolites in urine have been published, including the use of anion exchange in traditional solid phase extraction. This poster demonstrates an automated, fast, and highly sensitive extraction method for alcohol metabolites, ethyl sulfate (EtS) and ethyl glucuronide (EtG), in urine and oral fluid. The method uses only 50 µL of matrix for minimal sample volume, a four-fold total dilution factor, and 96 samples can be extracted in 10 minutes.

Objectives: Establish an automated and sensitive method for the analysis of alcohol metabolites in urine and oral fluid.

Methods: The automated liquid handling (ALH) platform is a Hamilton MICROLAB® NIMBUS96. The DPX tips contain strong anion exchange (SAX) sorbent. The sample is composed of 50 µL of matrix fortified at various concentrations, 10 µL of internal standard (Cerilliant (Round Rock, TX)), and 450 µL of acetonitrile. Well plates for conditioning, wash, and elution steps are pre-aliquoted for the extraction. Internal standards used were Ethyl-D5 sulfate sodium salt and Ethyl-D5-β-D-glucuronide.

The automated DPX XTR method follows a bind, wash, elute style protocol. The tips are first conditioned by aspirating and dispensing 750 µL of 50/50 methanol/acetonitrile to prepare the resin for sample binding. The ALH next picks up the DPX tips and aspirates and dispenses the 500 µL sample solution five times to maximize binding. The matrix interferents are then removed by aspirating and dispensing the wash solution, 750 µL of 50/50 methanol/acetonitrile, two times. The analytes of interest are eluted by aspirating and dispensing 500 µL of 2% hydrochloric acid in acetonitrile five times. The eluate is evaporated to dryness and is resuspended in 200 uL of HPLC water, resulting in a final four-fold dilution.

Analysis is performed on a Shimadzu LC40 paired with a SCIEX 6500+ mass spectrometer. The analytical LC column is a Restek Ultra Biphenyl, 3 um, 100 x 2.1 mm HPLC Column (PN 9109312). The choice of this biphenyl column allows for optimal separation of EtS and EtG from their interferents. Injection volume in this case is 5 µL, and mobile phases are 0.1% formic acid in water and 100% acetonitrile. This method was evaluated for linear dynamic range, extraction recovery, matrix effects, and limits of detection and quantification. Linearity is assessed by analyzing oral fluid and urine at seven concentration points ranging from 25-1500 ng/mL of both EtS and EtG in oral fluid and urine, covering common cutoffs associated with oral fluid and urine.

Results: Linear correlation coefficients for EtS and EtG were 0.99 and above in urine and oral fluid. In comparison to a centrifugation method with the same dilution factor, DPX XTR tips provide almost identical sensitivity of the analytes of interest in urine, further exhibiting the benefits of a DPX SPE method for this application. The common contaminant associated with EtS is removed by greater than 70%, allowing for seamless integration of the two normally co-eluting peaks and increased sensitivity in the quantification and qualification ions of EtS. The relevant cutoff values for EtS (25-100 ng/mL) and EtG (100-500 ng/mL) in urine and oral fluid are achieved with signal-to-noise above 10, further demonstrating the removal of matrix interferents.

Discussion: The use of DPX XTR Tips for the analysis of alcohol metabolites in urine and oral fluid is reproducible, sensitive, and aids in instrument cleanliness by removing matrix that can damage analytical systems. This method removes common interferents associated with alcohol metabolites in urine and oral fluid by more than 70% and allows for easy and sensitive downstream analysis.

P106

Development and Validation of a LC-MS/MS Method for the Quantitation of Synthetic Cathinones

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Introduction: Synthetic cathinones ("bath salts") are central nervous system stimulants that are derived from the Khat (*Catha edulis*) plant. While these products are typically labeled as "research chemicals", and "not for human consumption," they are often abused for their euphoric side effects. Common street names include "Boot", "Cloud Nine", and "White Lightening". The Drug Enforcement Agency (DEA) has scheduled several drugs within the class including eutylone, pentylone, N-Ethylpentylone, and all positional isomers to include N,N-Dimethylpentylone (Dipentylone). These compounds are the four most frequently encountered within the District of Columbia. This presentation focuses on the redevelopment of a quantitative method utilizing liquid chromatography- mass spectrometry-mass spectrometry (LC-MS/MS) for application in postmortem and human performance casework.

Objectives: The goal of this project was to re-develop the laboratory's synthetic cathinones quantitative method to include pertinent analytes, lower detection limits, and more efficient sample preparation in blood and urine. Analytes of interest include eutylone, pentylone, N-Ethylpentylone, and N,N-Dimethylpentylone.

Methods: A liquid-liquid extraction (LLE) was performed using 0.1 mL of authentic blood or urine, 0.5 mL of pH 9.3, 0.13 M sodium borate buffer and 0.01 mL of multicomponent deuterated internal standard. The samples were vortex-mixed followed by the addition of 1 mL of N-butyl chloride. The samples were then placed on a rotator for 5 minutes, sonicated for approximately 5 seconds, and then centrifuged for 5 minutes at 3500 rpms. The organic layer is transferred and fortified with 0.1 mL of 1% HCl before being evaporated under nitrogen (35°C). The extracts were reconstituted with 0.4 mL of starting mobile phase.

Data acquisition was performed on an Agilent 1290 Infinity II liquid chromatograph coupled to an Ultivo tandem mass spectrometer. Chromatographic separation was achieved using an Agilent Poroshell 120 Phenyl-Hexyl column (2.1 mm x 100 mm, 2.7 um) and an Agilent Poroshell 120 Phenyl-Hexyl guard column (2.1 mm x 5 mm, 2.7 um) as the stationary phase and a gradient of 0.1% formic acid in water and 0.1% formic acid in acetonitrile with a flow rate of 0.500 mL/min. The total run time was 10 minutes. Method validation was carried out in accordance with ASB Standard 036.

Results: Seven non-zero calibrators were chosen for all analytes, ranging from 10-500 ng/mL with a quadratic regression with 1/x weighting. The limit of detection was determined to be 5 ng/mL. Authentic matrix was spiked at concentrations of 20, 60 and 400 ng/mL to evaluate bias and precision, which were both within ± 20%. Furthermore, the laboratory established an equivalency between blood and urine. Thus, urine controls were not needed for routine casework. Interference studies, including matrix, internal standard, and other commonly encountered analytes, were performed with no known interferences detected. Ion suppression/enhancement, dilution integrity, and 72 hour sample stability were successfully evaluated.

Discussion: The laboratory was able to develop and validate an efficient LLE and LC-MS/MS method to quantitate select synthetic cathinones in routine casework. As drug trends are everchanging, the implementation of this method will allow the laboratory to monitor the prevalence of synthetic cathinones in both Postmortem and Human Performance cases.

P107

In vitro evaluation of the toxicity of synthetic psychoactive cathinones

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Background: In 2021, a staggering 33,000 American lives were lost to psychostimulant overdoses, accounting for over 30% of all drug overdoses that year (CDC, 2022). Synthetic psychoactive cathinones (SPCs) are novel psychoactive substances with effects similar to cocaine, methamphetamine, and methylenedioxymethamphetamine (MDMA). SPCs are of great concern because of their abuse liability and potential for adverse effects. There is little knowledge of how amphetamine or cathinone overdose occurs mechanistically. Because of this the adverse effects are treated symptomatically instead of targeting underlying mechanisms. Clinical reports suggest that stimulant overdose mainly affects the heart, the brain, and the liver. *In vitro* approaches can evaluate the underlying mechanisms of the toxic effects of SPCs in specific cell types.

Methods: HepG2 (hepatic), AC-16 (cardiac), and SH-SY5Y (neural) cells were exposed to each amphetamine or SPC. The compounds evaluated were MDMA, methamphetamine, amphetamine, methylone, ethylone, dimethylone, butylone, eutylone, dibutylone, pentylone and MDPV. Half-maximal inhibitory concentration (IC50) values were determined in a cell viability assay using 3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide (MTT). Adenosine triphosphate (ATP) was assessed using an assay that quantifies the number of viable cells by measuring the levels ATP. HepG2 cells were cultured in glucose and galactose media to determine differences in ATP levels under greater oxidative metabolic demand. Reactive oxygen species (ROS) were assessed by a bioluminescent assay in HepG2 and SH-SY5Y cells based on results of MTT assay for concentration ranges. The levels of glutathione (GSH) were also assessed via a bioluminescent assay in HepG2 cells based on results of the MTT assay for concentration ranges.

Results: Significant ROS production was seen for methylone, butylone and pentylone in both HepG2 and SH-SY5Y cell lines but not MDMA, dimethylone, eutylone, dibutylone and MDPV. Ethylone also showed significant ROS production but only in HepG2 cells. A significant decrease in GSH was seen for methylone and butylone, but a significant increase in GSH was seen for ethylone, dibutylone and pentylone. The levels of ATP between HepG2 cells in glucose cultures compared to galactose cultures differed after exposure to methylone. Higher IC50 values were seen for reduction in ATP levels compared to the IC50 values found via the MTT assay. **Conclusions:** This toxicity evaluation of SPCs gives more insight into how they produce their toxic effects. Initially we expected that all these drugs that are structured similarly would produce their effects the same way. However, our results show that SPCs with smaller side chains produce a significant increase of ROS compared to the ones with larger side chains. This indicates that the initiation of cellular death is different depending on these molecular features, suggesting a structure activity relationship for toxic actions.

P108

Distribution of Protonitazene, Bromazolam, and their Metabolites in a Fatal Overdose: A Case Study

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Introduction: While fentanyl saturates the drug market, designer benzodiazepines and nitazenes are increasingly encountered in the Northeast Ohio region. Recent trends show an increased prevalence of polydrug use involving the combination of benzodiazepines and opioids, creating significant public health concernS[1]. NPS tissue distribution data are scarce because of the dynamic drug market, complicating forensic and clinical toxicology casework.

Objectives: We present a unique case report of a 21-year-old male who presented to the hospital following a suspected overdose and expired approximately 50 minutes after his arrival. The decedent had a history of anxiety, post-traumatic stress disorder, and unspecified drug abuse. Postmortem toxicology testing identified protonitazene, bromazolam, 7-aminoclonazepam (9.4 ± 1.5 µg/L), and Δ9-THC. No admission specimens were obtained for testing. We aimed to investigate the postmortem distribution of protonitazene, *N*-desethylprotonitazene, bromazolam, and α-hydroxybromazolam in the femoral blood, heart blood, urine, vitreous humor, liver, and brain (basal striatum).

Methods: The target analytes were detected by liquid chromatography-tandem mass spectrometry (LC-MS/MS) following solid phase extraction, using the method of standard addition (MSA) for calibration. Known amounts of a reference standard were added to three aliquots of a case specimen. The concentration of a fourth unspiked aliquot was then determined by extrapolation. The internal standard was fentanyl-D5 for protonitazene/*N*-desethylprotonitazene and α-hydroxymidazolam-D4 for bromazolam/α-hydroxybromazolam. Specimens were extracted and analyzed with in-house fentanyl/analogue[2] and benzodiazepine methods[3].

Results: The MSA-derived femoral blood concentration of bromazolam (2,634 µg/L) was corroborated by a reference laboratory (3,100 µg/L). In comparison the heart blood concentration of bromazolam was 2,395 µg/L. In the femoral and heart blood, α-hydroxybromazolam concentrations were 157 and 120 µg/L. However, the bromazolam concentrations in the urine, liver, and brain were >6000 µg/L or µg/kg. Vitreous humor concentrations were lower (1,187 µg/L). α-hydroxybromazolam concentrations were 32 µg/L, 531 µg/kg, and 8,833 µg/kg in the vitreous humor, liver, and brain.

Protonitazene concentrations in the femoral and heart blood were 0.14 and 0.10 µg/L. Vitreous humor, liver, and brain concentrations were higher than in the blood. Surprisingly, the urinary protonitazene and *N*-desethylprotonitazene concentrations were both 1.7 µg/L. N-desethylprotonitazene femoral and heart blood concentrations were <0.10 µg/L.

Discussion/Conclusion: This report is the first of postmortem distribution data for α-hydroxybromazolam and *N*-desethyl protonitazene. The cause of death in this case was directly linked to NPS, providing a valuable reference of postmortem concentrations and preliminary insights into toxicokinetics. For example, high brain concentrations may indicate a strong tendency to cross the blood-brain barrier, increasing their toxicity potential. Our findings align with previous synthetic opioid data, which also report high concentrations in the brain, blood, and vitreous humor[4]. Vitreous humor may be valuable for postmortem protonitazene and *N*-desethylprotonitazene detection due to its high observed concentrations. Future research should evaluate cases with more complete case histories and scene information, including the mode of administration and survivability time between ingestion and death, to evaluate the generalizability of these results.

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P109

Impaired Driving Surveillance in Arkansas: A Collaboration between the Arkansas State Crime Laboratory and Glen F. Baker Public Health Laboratory.

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Introduction: Alcohol has primarily been associated with impaired driving, and its effects on drivers are well understood. However, polysubstance use among impaired drivers has become more prevalent. Studies have shown that a significant percentage of impaired drivers test positive for both alcohol and drugs, and that this population has a significant increase in crash risk [1]. Thus, the National Safety Council has recommended that forensic toxicology laboratories regularly test blood from suspected impaired drivers for impairing drugs and their metabolites. Unfortunately, many forensic laboratories are limited in personnel, budget, and resources and are unable to undertake comprehensive drug testing for each impaired driving case. As a result, stop limit testing may be implemented as standard procedure if a driver's blood alcohol concentration (BAC) is measured above a specific cutoff, usually at or above the *per se* limit, and drug testing will not be performed for these cases as impairment could be demonstrated with the BAC result alone. Missing data on the driving population's drug usage has made it difficult to assess the prevalence of drugged driving and the crash risk associated with an individual that has drugs or combinations of drugs in their system.

To address the gaps in drug use data among impaired drivers, the Arkansas State Crime Laboratory (ASCL) and Glen F. Baker Public Health Laboratory (GFBPHL) have collaborated to begin an Impaired Driving Surveillance Project in the state of Arkansas. De-identified blood samples from suspected impaired drivers were provided to the GFBPHL by the ASCL for comprehensive drug screening. By providing de-identified samples, the GFBPHL can expedite sample testing and reporting, which will provide up-to-date information on a rapidly shifting drug landscape to the ASCL, law enforcement, and healthcare providers.

Objectives: The primary objective of the Impaired Driving Surveillance Project will be to provide critical data to determine the prevalence of polysubstance impaired driving in Arkansas. The authors also will aim to demonstrate a roadmap to successful and productive collaborations between state laboratories that further the mission of each's respective agency.

Methods: The ASCL provided the GFBPHL with de-identified blood samples from impaired driving casework. Samples were processed by supported liquid extraction (SLE) and analyzed via a Thermo Scientific Orbitrap Exploris 480 to acquire full-scan, high-resolution MS and unit-resolution MS2 data. Reference material for each compound of interest was analyzed to create an in-house MS2 spectral library which was used to assist in identification and confirmation of compounds. Multi-drug and cannabinoid methods were utilized and consisted of targeted compounds selected from the Tier I and Tier II recommendations of the National Safety Council's Alcohol, Drugs, and Impairment Division (NSC-ADID) [2].

Results: The project commenced in June 2024. This presentation will include data collected between June and September 2024, offering insights and preliminary findings of the prevalence of polysubstance impaired driving in the State of Arkansas from this period.

Discussion: Discussion will focus on the implications of the results with respect to how the ASCL, law enforcement, and healthcare providers can use these data to better inform policymakers and stakeholders to manage impaired driving issues facing Arkansas more effectively. The discussion will also promote collaboration and outreach between state forensic and public health laboratories.

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P110

Evaluating the performance of commercial nitazene immunoassay test strips for drug checking applications

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Introduction: Recently, 2-benzylbenzimidazole 'nitazene' opioids have emerged on the recreational drug market as new psychoactive substances. With potencies often exceeding that of fentanyl, nitazene use is particularly dangerous, as evidenced by the increasing number of overdose deaths linked to this highly dangerous class of new synthetic opioids. Furthermore, nitazene-adulterated street drug samples are also increasingly being detected, with nitazenes found to be mixed with heroin or being found in counterfeit non-opioid (e.g., benzodiazepine, ecstasy) and opioid (e.g., oxycodone) tablets. This has resulted in intoxications where drug users were unaware that they had taken a nitazene. These recent developments highlight the need for different strategies such as harm reduction approaches to address the growing public health risk posed by nitazenes. Similar to the availability of fentanyl test strips, lateral flow immunoassay nitazene test strips (NTS) recently became available in Q1 2024, with the aim to allow users to rapidly determine whether or not a drug sample contains a nitazene. While the manufacturer specifies that the NTS do not cross-react with other (non-)opioid drugs or with common cutting agents, the development of these NTS involved testing with only a limited number of nitazenes. Therefore, comprehensive information on the cross-reactivity with other nitazenes and real-word applicability of these NTS for drug checking purposes is lacking.

Objectives: The aim of this study was to independently assess the performance of the first publicly available nitazene test strips (NTS) for drug checking purposes. The cross-reactivity of the NTS with other nitazenes and their ability to detect the presence of a nitazene in authentic drug samples was investigated.

Methods: The limit of detection (LOD) for isotonitazene, the target analyte with a reported cut-off of 2000 ng/mL, was determined using two manufacturing lots of NTS by conducting 6 replicate measurements of 6 different concentrations of isotonitazene ranging from 500 to 3000 ng/mL. Reference standards of 33 different nitazenes were dissolved in Milli-Q water, resulting in solutions of 1.8% solvent in water. To evaluate NTS cross-reactivity, these drug dilutions were screened on the BTNX Rapid Response™ nitazene test strips (BTNX Inc, Ontario, Canada) in least at two concentrations ranging from 1000 to 9000 ng/mL. Real-world NTS applicability was evaluated with 6 different authentic drug samples (metonitazene, protonitazene, isotonitazene, butonitazene, *N*-pyrrolidino etonitazene, and *N*-piperidinyl etonitazene powder samples).

Results: The LOD for isotonitazene was found to be 2000 or 3000 ng/mL, depending on the lot. Out of the 33 screened nitazenes, 24 compounds were detectable at or below 3000 ng/mL, and 9 compounds were not detectable at 9000 ng/mL. A structural analysis of the evaluated nitazenes indicated that either modification or removal of the nitro group at the 5-position of the benzimidazole ring, or lengthening the linker between the two aromatic rings, generally compromises detection by NTS. For all 6 evaluated authentic drug samples, the NTS could accurately identify the presence of a nitazene, with no observed false negatives.

Discussion/Conclusion: The evaluation of Rapid Response™ NTS in this study provides a better understanding of their applicability for drug checking purposes. Our results indicate that these NTS can alert to the presence of most nitazenes that have emerged on recreational drug markets worldwide, with concentrations in the low µg/mL range being detectable. However, it should be noted that 'des'-nitazenes (analogs lacking the 5-nitro group) may yield false negative results due to low cross-reactivity. Theoretically, our findings suggest that detection of a nitazene down to a level of 0.9% to 0.1% by weight may be obtainable. While this is lower than the recently reported 11% protonitazene content in a powder mis-sold as heroin, the unregulated nature of the drug market essentially implies that any content is possible. Considering the high potency of many nitazenes, the presence of even trace amounts in drug samples, which could be below the cut-off for detection with NTS, may still lead to dangerous opioid effects. In addition, other factors such as specificity, batch-to-batch variability in performance, solubility, and different experimental conditions should be taken into account. Despite these limitations, these commercial NTS can serve as important overdose prevention tools for drug users, where a positive result from a suspicious drug sample would be indicative of adulteration with a nitazene.

P111

Identification of 15 Urinary Biomarkers for Cannabis Exposure Across Various Cannabis Users

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Background/Introduction: Marijuana is the most used illicit drug in the United States, with legislation varying from state to state. These legal differences necessitate distinguishing between various types of users, such as medicinal, recreational, chronic, or acute. Urine is commonly used to test for marijuana in such cases, as its lipophilic nature allows for a wider detection window and the collection method is noninvasive. When marijuana is consumed, it is stored in fat and gradually released over time through urine as metabolites. This results in a longer detection window compared to other matrices such as blood or saliva, making urine an effective medium for detecting the drug and its metabolites.

Currently forensic toxicological tests focus on identifying delta-9-tetrahydrocannabinol (Δ^9-THC), which alone is insufficient for differentiating between different types of users, and primarily serves to confirm the presence of marijuana. This limitation makes it challenging to accurately assess marijuana usage and its implications in various contexts, such as law enforcement and medical evaluations. Therefore, targeting additional cannabinoids is necessary to gain more comprehensive information, aiding in accurate assessments and investigations.

Objectives: This research focuses on creating a quick and efficient method to quantify cannabinoids and their metabolites in urine utilizing an optimized liquid chromatography triple quadrupole mass spectrometer (LC-QqQ-MS) method in dynamic multiple reaction monitoring (dMRM) mode. The developed and optimized method will be used to analyze authentic urine samples from both cannabis smokers and non-smokers. Results from a questionnaire given to the human cohort, in which participants indicate whether they are chronic, acute, medicinal, or recreational users, will be paired with the urine analysis data. Based on these combined results, multivariable statistical analyses will be performed to categorize the various biomarkers and link them to user types.

Methods: A total of 15 cannabinoids and their metabolites were targeted for analysis including 12 internal standards. An Agilent 1290 Infinity UHPLC coupled with a 6470 Lc-QqQ-MS with ESI in both positive and negative mode was employed. The chromatographic column used was a Zorbax 120 EC-C18 column (3.0 x 100 mm, 1.8 µm) paired with a step gradient using 75% Acetonitrile (ACN) and 25% Methanol (MeOH) in .1% formic acid (FA) and 5mM ammonium formate (AF) in 0.1 %FA in water. In addition, a cannabinoid mix was created for the 15 standards and a separate mix for the 12 deuterated internal standards yielding a concentration of 100ppb for each mix.

Results: Using the method on the cannabinoid mix with 15 standards, each compound was chromatographically separated and identified, with a total run time of 13 minutes for each injection. However, when comparing the abundance of the standards, the acids like 7-Carboxy-cannabidiol (7-COOH-CBD) and 11-nor-9-carboxy-THCV (Δ^9-THCV-COOH) were significantly lower than the other compounds in both positive and negative polarity. Similarly, when applying this method to the cannabinoid mix with 12 deuterated standards, each compound was chromatographically separated and identified. However, as before, the abundance of acids such as 7-COOH-CBD-D_3 and Δ^9-THCV-COOH-D_3 was low.

Conclusion/Discussion: Overall, this method successfully separated and identified each cannabinoid, though the acidic cannabinoids were present in lower abundance compared to others. Therefore, future optimization is necessary to ensure a higher abundance of the acids. Lastly authentic urine samples obtained from a human cohort of cannabis and non-cannabis users will be analyzed using the established method paired with extraction techniques such as dilute-and-shoot and supported liquid extraction.

P112

The Comparison of Whole Blood and Vitreous Fluid Drug Findings in Fifty Postmortem Cases.

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Introduction: Vitreous fluid is commonly tested to determine the absorption state of ethanol and to determine heroin exposure or recent cocaine use. It can also be tested as a second specimen to confirm drug findings in another matrix. Depending upon the circumstances of a death, vitreous fluid may be the best or only specimen available for toxicology testing. Data regarding the presence and concentration of drugs in vitreous fluid as compared to whole blood is limited.

Objectives: Due to the properties and aqueous nature of vitreous fluid, could it be used for general drug screening in the absence of other matrices? Would some drugs be missed? Confirmed drug findings in fifty cases were compared between blood, vitreous fluid, and urine when available. These data are important to help determine if a specific drug would be expected to be detected in vitreous fluid. Knowledge of the limitations of using vitreous fluid as a matrix in which to perform general drug screening is necessary for the proper interpretation of the results.

Methods: The blood, vitreous fluid, and urine were screened by liquid chromatography-quadrupole time of flight mass spectrometry (LC-QTOF-MS) for approximately 350 drugs in the following drug classes: amphetamines, analgesics, anesthetics, anticholinergics, anticonvulsants, anticoagulants, antidepressants, antidiabetics, antifungals, antihistamines, antipsychotics, barbiturates, benzodiazepines, cannabinoids, cardiovascular agents, designer opioids, gastrointestinal medications, hallucinogens, muscle relaxants, novel psychoactive substances, opioid analgesics, sedatives, stimulants, synthetic cannabinoids, and urological agents. Blood, vitreous fluid, and urine were sent to confirmation for those drugs that screened positive. The screening and confirmation methods were fully validated in all three matrices. In some confirmation methods the vitreous fluid or blood only validated for qualitative confirmation.

Results: Drugs that confirmed positive were members of the following classes: anesthetics, anticoagulants, some antidepressants, some antipsychotics, anticonvulsants, antihistamines, some benzodiazepines, some designer opioids, some muscle relaxants, opioids, cardiovascular agents, stimulants, sympathomimetic amines, and some urological agents. Drugs and drug classes that confirmed in blood but were not detected in vitreous fluid were THC*, THC-OH, THC-COOH*, xylazine, warfarin, some benzodiazepines, some antipsychotics, some antidepressants, some urological agents, some muscle relaxants, and analgesics. Some drugs only had one or a few occurrences therefore more data for these drugs are needed.

Discussion: *There was one case with a very high concentration of THC (252 ng/mL) and THC-COOH (597 ng/mL) which confirmed for THC and THC-COOH in vitreous fluid just above the reporting limits of 1 ng/mL and 3 ng/mL, respectively. This case also confirmed for THC-OH (82.7 ng/mL) in the blood but was not detected in the vitreous fluid with a reporting limit of 3 ng/mL. All other cases with THC-COOH confirmed in the blood did not screen positive in vitreous fluid with a cut-off of 15 ng/mL. Commonly encountered illicit drugs did confirm in vitreous fluid. In fact, a lingering opioid death may be able to be determined by comparing blood and vitreous fluid concentrations. Two cases involving fentanyl will be discussed as an example with blood fentanyl concentrations of 1 and 1.9 ng/mL, and vitreous fluid fentanyl concentrations of 11.7 and 7.8 ng/mL, respectively. Vitreous fluid can be useful in the detection of some drugs but not all drugs. In an acute overdose, drugs may not have had enough time to pass into the vitreous fluid before death occurred. Also drugs at lower concentrations in blood may not be detectable in vitreous fluid, as well as those drugs that are lipophilic like benzodiazepines. Vitreous fluid has its place in toxicology testing, however due to its' limitations, using it as a specimen for general drug screening should be avoided.

P113

A Quantitative Separation Workflow for Δ8, Δ9 & Δ10-THC Analytes and their Respective Metabolites Extracted from Whole Blood Using Enhanced Matrix Removal and LC/MS/MS.

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Introduction: The ability to isolate and analyze individual structural isomers of psychoactive THC analytes and their respective metabolites from non-psychoactive structural isomers such as CBD has become necessary in recent years for a variety of reasons. One reason is the toxicological analysis of blood samples in suspected DUI scenarios. This study describes the extraction, separation, and quantification of delta8, delta9, and delta10-THC, and their respective carboxy- and hydroxy- metabolites, in whole blood samples. Separation from each other, and from other isobaric interferences such as Cannabidiol (CBD) and Exo-THC, was achieved using LC/MS/MS.

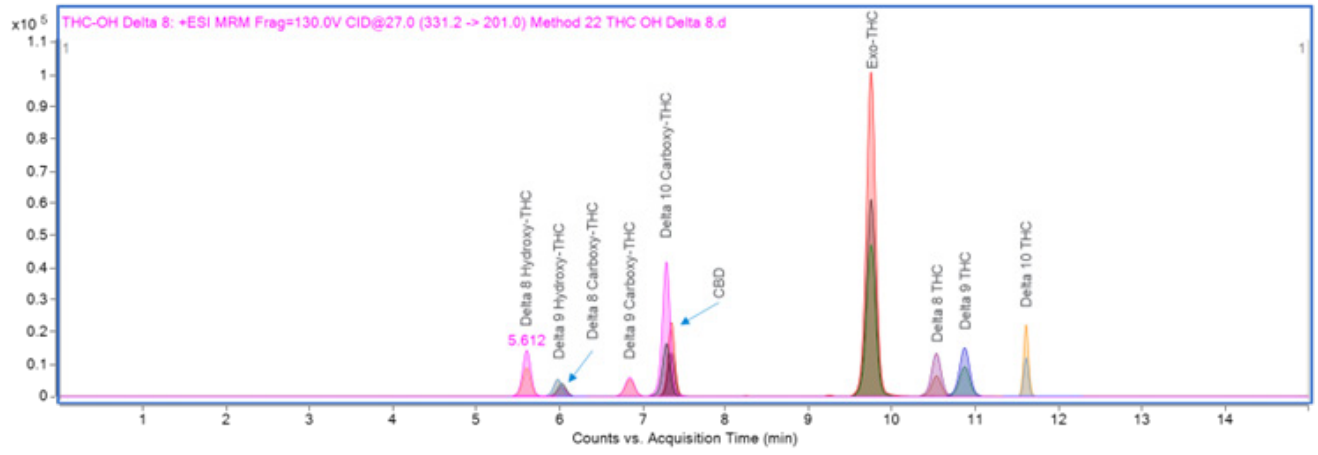
Objectives: To demonstrate a workflow to extract, separate, identify, and quantify THC isomers and their respective carboxy- and hydroxy- metabolites from whole blood using LC/MS/MS in a single 15 minute analysis.

Methods: Δ8, Δ9 & Δ10-THC analytes and their respective carboxy- and hydroxy-THC metabolites were extracted from whole blood using a modified routine utilizing Captiva Enhanced Matrix Removal (EMR) cartridges. The LC/MS/MS analysis method was developed and performed using an Agilent Technologies 1290 HPLC system and 6475 TQ Mass Spectrometer in positive ion mode. Baseline chromatographic separation of all potential isobaric analytes and interferences was achieved in a single analysis using an aqueous 0.1% formic acid/methanol mobile phase gradient through an Agilent Poroshell 120 PFP column (100 x 2.1mm, 2.7um) with an analysis time of 15 minutes.

Results: Analyte recoveries between 70-130% were achieved for the whole blood extraction workflow using a Captiva EMR-Lipid extraction routine. Low ng/mL sensitivity for each analyte will be shown together with their respective quantitative linear ranges.

Baseline chromatographic separation of analytes and their respective carboxy- and hydroxy-THC metabolites will be demonstrated in a single analysis injection and further separation from other potential isobaric interferences such as CBD will be shown.

Example chromatogram:



Discussion: Herein, we demonstrated that it is possible to chromatographically separate Δ8, Δ9 & Δ10-THC analytes and their respective carboxy- and hydroxy-THC metabolites with baseline separation using a poroshell 120 PFP column chem-

istry of 100 x 2.1mm dimensions in a single sample analysis. Potential isobaric non-psychoactive interferences such as CBD and Exo-THC were also separated as part of the aim of the analysis.

Low ng/mL sensitivity for all analytes of interest was achieved with good linear dynamic range using this methodology using a 15 minute analysis time.

Analyte recoveries of between 70-130% were achieved in whole blood samples using an extraction workflow based around Captiva EMR-Lipid cartridges.

P114

A Unified Liquid Chromatography-Tandem Mass Spectrometry Method for the Quantification of Seven Ethanol Biomarkers in Blood and Oral Fluid

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Background and aim: Ethyl glucuronide (EtG) and ethyl sulfate (EtS), non-oxidative metabolites of ethanol, are the most common biomarkers in clinical/forensic toxicology to assess ethanol consumption. Other biomarkers include 5-hydroxy-indolacetic acid (HIAA), 5-hydroxytryptophol (HTOL), and 5-hydroxytryptophol-β-D-glucuronide (GTOL), which stem from serotonin metabolism in the presence of ethanol. Phosphatidylethanol (PEth) is an abnormal phospholipid, with multiple possible isoforms, formed in the presence of ethanol. While most of these ethanol biomarkers are reportedly cleared from the body in days, the window of detection for PEth has been reported as days to months depending on the manner of drinking (i.e., moderate, chronic). The understanding of the formation and detection of these these seven analytes in blood, urine, and oral fluid varies, depending on analyte and matrix.

While blood is the specimen gold standard for toxicology testing, oral fluid (OF) is gaining interest as a replacement. The aim of this study was to develop a unified method incorporating seven biomarkers, including two of the most prevalent PEth isoforms, to facilitate interpretation of the timeline and dose of prior ethanol consumption, and investigate the relationship between biomarkers in blood and oral fluid using samples collected during a clinical study. Presented is the evolution of the method development, associated challenges and limitations, and clinical results.

Methods: Several columns, mobile phases, and instrument parameters were investigated over 3 years to develop an optimum method that enabled the identification and quantitation of seven analytes simultaneously. A method using a Shimadzu LCMS-8050 equipped with a Luna Omega Sugar column (100 x 2.1 mm, 3 µm) and a gradient method using mobile phases of 25 mM ammonium formate with 0.2% formic acid (A) and 85:15 acetonitrile:isopropanol with 0.1% formic acid (B) was used. Positive multiple reaction monitoring (MRM) mode was used for HIAA and HTOL data collection, while negative MRM was used for the remaining analytes. Calibration ranges were: 10-1000 ng/mL for HTOL, GTOL, PEth 16:0-18:1 and PEth 16:0-18:2; 50-5000 ng/mL for EtG and EtS; and 100-10000 ng/mL for HIAA.

Blood and OF were collected from seven participants during a clinical study at predetermined timepoints across the pharmacokinetic time course. Blood was collected in grey top tubes, and OF was collected using Quantisal devices. Samples were stored at -80°C until analysis. Several iterations of sample extractions (liquid-liquid, solid phase, and supported liquid) were investigated in an effort to find a single method applicable to the extraction of all seven analytes in all three matrices. The final extraction method was ice cold acetonitrile added dropwise to the sample and internal standard while vortexing, followed by sample dry down and reconstitution.

Results: GTOL and HTOL were not detected in blood or OF. EtG, HIAA, and the two PEth isoforms were not detected in OF. Determined analyte ranges by matrix are presented in the table below.

	Detected Range (ng/mL)	
	Blood	Oral Fluid
EtG	71-563	ND
EtS	52-335	51-157
GTOL	ND	ND
HIAA*	103-415	ND
HTOL	ND	ND
PEth 18:1	10-150	ND
PEth 18:2	10-181	ND

*Endogenous

Conclusions: Vaping a 20% ethanol e-liquid alone did not produce detectable concentrations of any biomarker. EtG and EtS were present in all blood samples following oral alcohol consumption. One participant had both PEth isoforms present in blood, including the pre-session baseline collection, in three of the four sessions, indicating previous ethanol consumption. Blood and oral fluid results will be compared to determine any trends. A unified extraction and analytical method will help streamline laboratory operations, while expanding the interpretive value of the analytical results.

P116

Characterization of oral nicotine pouches: An evaluation of biorelevant in vitro release, pH, and nicotine content

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Introduction: The National Youth Tobacco Survey reported in 2023 that 1 in 100 middle school students and 2 in 100 high school students have used oral nicotine pouches (ONPs) in the past 30 days. Despite the increase in popularity amongst youth, these ONPs remain to be less regulated compared to traditional smokeless tobacco or combustible tobacco products. Thus, it is important to characterize these products for potential exposure and toxicity.

Objectives: The objective of this study is to characterize the in vitro release, pH, and nicotine content of commercial ONPs using a novel in vitro device, the bidirectional transmucosal apparatus (BTA), to simulate the oral cavity.

Methods: Commercial ONPs On!, Velo Max, Dryft, and Zyn, containing nicotine ranging from 2 to 8 mg, were evaluated for cumulative and amount of nicotine release using the validated BTA device. Samples were collected at various time points up to 90 minutes. Quantitation was performed on a Waters Acquity H-Class HPLC-PDA with a Thermo Scientific Hypersil GOLD Phenyl column (5 µm, 150 x 4.6 mm). For pH determination, ONP samples were prepared in water or artificial saliva, and measurements were conducted after 5, 15, and 30 minutes of stirring.

Results: Cumulative release across the ONP products was greater than 85%, achieved within 20 min, and acceptable under the United States Pharmacopeia guidelines. Maximum release of nicotine across all ONP products occurred within 30 minutes. The pH of ONP products prepared in water ranged from 5.6 to 8.7. The pH of ONP products prepared in artificial saliva ranged from 6.8 to 8.0.

Discussion: These products achieved maximum nicotine release within 30 minutes. The pH of the ONPs prepared in water remained constant over sampling time. In artificial saliva, the pH of the On!, Velo Max, and Dryft ONPs slightly increased whereas the pH of Zyn continued to trend upwards over time, suggesting a time-independent impact on the pH of Zyn which may affect the percent free base nicotine available.

P117

Validation of an analytical method for quantitation of Xylazine in human umbilical cord tissue

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Introduction/Objective: Xylazine is a sedative approved for veterinary medicine but not approved for human consumption. Xylazine has commonly been found as an adulterant, unbeknownst to the user, but can also be abused as a drug in its own right. Negative side effects of xylazine include CNS and respiratory depression, hypotension, hypothermia, high blood glucose levels, miosis, hypotension, and necrotic skin ulcerations that can lead to amputation. According to the DEA, there were 149 reported cases involving xylazine in 2015, but that number jumped to 8,938 in 2021. Little is known about the frequency and side effects of in utero exposure to xylazine. This poster aims to share a validated method for the analysis of xylazine in umbilical cord tissue.

Methods: The umbilical cord (0.5g) is weighed and then homogenized in acetonitrile. The sample is centrifuged and internal standard (d6-xylazine) is added. The supernatant is evaporated and reconstituted with a pH 6 phosphate buffer. The samples were loaded onto a Clean Screen DAU extraction column, which was previously conditioned with 3 milliliters of methanol, deionized water, and pH 6 phosphate buffer, respectively. The solid phase cartridges were then washed with 3 milliliters of deionized water then 0.1 M hydrochloric acid and finally 1 milliliter of hexane. Samples were eluted with 3 mL of 80:20 methylene chloride : isopropanol with 2% ammonium hydroxide. After evaporation at 40°C under nitrogen, samples were reconstituted with 50 µL of 6% acetonitrile in water with 0.1% formic acid. Samples were then analyzed on a Liquid Chromatography Tandem Mass Spectrometer (LC-MS/MS).

The LC-MS/MS included an Agilent 1200 liquid chromatography system and a Sciex 5500 Trip quad tandem mass spectrometer in positive ionization mode. Separation was achieved using a Phenomenex 50 mm x 2.0 mm Synergi Polar-RP column with 2 µm particle size. Mobile phase A was deionized water with 0.1% formic acid and mobile phase B was acetonitrile with 0.1% formic acid. The following transitions were monitored xylazine: 221.0->90.2, 164.0 and for d6 xylazine: 226.8->90.1, 170.0.

Results: The method was fully validated according to ANSI/ASB Standard 036, Standard Practices for Method Validation in Forensic Toxicology. The linear range was 0.2 ng/g to 10 ng/g with a cutoff of 1 ng/g and a limit of detection of 0.02 ng/g. Precision and bias were acceptable, <20% and extracts were stable up to 7 days. Matrix effect showed ion suppression less than 25% and no interferences were observed.

Discussion/Conclusion: Xylazine is a growing threat in the United States and is quickly becoming a concern in the neonatal population. Accurate identification of prenatal exposure is crucial for appropriate neonatal treatment for the best possible outcome.

P118

Application of Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS) for the Identification and Determination of Narcotic Substances in Human Nail Samples from Jordanian Subjects

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Introduction: Nails are keratinized structures that can be used to determine the presence of narcotic substances as an alternative to traditional biological samples such as blood, hair, and urine. Nails can deposit substances in a stable manner for lengthy periods of time, offering retrospective evidence on the use of illicit drugs and pharmaceuticals. Many aspects such as the larger detection window, and ease of non-invasive collection make nails a powerful matrix for toxicology and pharmacology studies. The present study aims to measure the presence of five drugs in human nail samples of admitted drug users.

Objectives: The aim of the study was to determine the possibility of identifying amphetamine, methamphetamine, tetrahydrocannabinol, cocaine, and alprazolam in the Jordanian drug market by examining their presence in fingernails using liquid chromatography tandem quadrupole mass spectrometry (LC-MS/MS).

Methods: Fingernail samples were taken from 33 Jordanian volunteers who had a self-reported history of drug addiction. Samples were collected by clippings from all ten fingers. Samples were tested for amphetamine, methamphetamine, tetrahydrocannabinol, cocaine, and alprazolam. The nail sample extraction procedure was performed according to a previously validated and peer reviewed method. Nail samples were decontaminated by three sequential washing steps with 2 ml dichloromethane (vortex mixing for 2 min). Decontaminated samples were left to dry at 80°C, 30mg of the nail were weighed and crushed by using a ball mill. Internal standard solution was added and incubated with horizontal agitation. Following extraction, the samples were analyzed by LC-MSMS

Results: The distribution of amphetamine, methamphetamine, tetrahydrocannabinol, cocaine, and alprazolam was highly varied among the 33 nail samples. Moreover, the concentrations of the same substance detected in the samples differ considerably. Amphetamine was detected in 29 nail samples where the highest concentration was 45.3 ng/mg and the lowest concentration was 0.07 ng/mg. Twenty-two nail samples were found to contain methamphetamine with the highest concentration being 11.8 ng/mg. Tetrahydrocannabinol was detected in 4 nail samples with the highest concentration being 0.78 ng/mg. Alprazolam was present in 35 samples but could be quantified only in three samples where the highest concentration was 2.0 ng/mg.

Conclusion/Discussion: There is a lack of statistics on substance usage in Jordan because there is no national epidemiological data gathering system for alcohol or drugs. According to the Anti-Narcotics Department (AND), the number of individuals with substance use disorders is less than 1% of the Jordanian population. This research gives an Indication of the distribution of narcotic substances among drug abusers in Jordan. It has been confirmed that methamphetamine and amphetamine are most widespread among these 33 Jordanian substance users with 87.9% and 66.7%, respectively, of nail samples. Tetrahydrocannabinol was detected in 12.1% of the nail samples. Alprazolam was present in 9.1% of nail samples while cocaine was present in only 3.03%.

LC-MS/MS is a high-performance, sensitive, robust, quick, and precise technique that was demonstrated to be an effective method for detecting a wide range of drugs in nail samples. Nails were shown to be an acceptable alternative biological matrix for providing additional data in a forensic toxicological context. The results of the drug test in the nails were a helpful factor in demonstrating drug use for subjects suspected of drug use.

P119

Detecting Novel Psychoactive Substances Through the Use of a Surveillance Library: a 2023 Review

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Introduction: Novel psychoactive substances (NPS) in the illicit drug supply have evolved dramatically and present major challenges for forensic toxicology laboratories. Their rapid emergence, proliferation, variable lifespans, and potential for significant harm to consumers present a need for laboratories to keep up to date with trends and develop testing capable of detecting these substances. High resolution mass spectrometry (HRMS) instruments, such as liquid chromatography/time-of-flight mass spectrometry (LC-TOF/MS), coupled with a dynamic surveillance library are increasingly utilized for this purpose. The application of a surveillance library in this manner allows for quick identification of new substances that may be present in a biological specimen and can be done in real-time or retrospectively.

Objective: To promptly detect and identify NPS in forensic casework and explore current NPS trends, with specific review of data collected from authentic specimens submitted from toxicology casework analyzed in 2023.

Methods: LC-TOF/MS was used to screen blood, serum, and urine samples of authentic forensic casework. The data generated from these samples were processed simultaneously with a routine test scope and a surveillance library. The surveillance library consists of roughly 60 compounds, most of which are NPS, and is routinely updated with emerging drugs. The included compounds are designated as either Type 1—meaning confirmation testing is available for further testing at the laboratory—or Type 2—which are added for monitoring purposes. Any finding is evaluated by a toxicologist who would then determine if further testing is warranted based on case history, other findings, and the submitting agency's approval.

Results: In 2023, there were 3860 samples from 3848 cases in which one or more NPS findings were detected through the surveillance library at the laboratory. In total, there were 4693 verified findings, with the designer benzodiazepine bromazolam accounting for over 60% of the detections. Several nitazene analogs were also detected, with metonitazene being the most prevalent with 173 detections. Other nitazene analogs identified included isotonitazene/protonitazene (which would be differentiated during confirmation testing), n-pyrrolidino protonitazene, n-pyrrolidino etonitazene, and n-desethyl isotonitazene. Additional NPS routinely detected through this process included other synthetic opioids, such as brorphine with 47 detections, other designer benzodiazepines, such as 8-aminoclonazolam with 243 detections, synthetic stimulants, such as N,N-dimethylpentylone with 226 detections, and a tricyclic antidepressant misused for its opioid-like effects, tianeptine with 59 detections. Overall, the laboratory had an approximate confirmation rate of 92% when additional testing for these substances was pursued.

Discussion: The illicit drug market is constantly evolving, with new substances being introduced at alarming rates. This phenomenon creates significant challenges for forensic toxicology laboratories but also endangers public health. Because of the dangerous nature of these compounds, it is essential to develop testing capable of detecting them and do so in a timely manner. The use of a dynamic surveillance library coupled to HRMS instrumentation permits quick and easy identification of NPS in forensic casework. This process also assists in monitoring emerging trends as confirmation testing is not necessary for tracking these compounds' prevalence. However, additional testing is not always approved by the submitting agency. For example, in casework submitted for LC-TOF/MS screening in 2023, additional confirmation testing was pursued roughly 70% of the time, which leads to underestimation and underreporting of these substances in authentic casework.

P120

Evaluation of Methamphetamine Positivity in the DUID Population (2018 - 2023)

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Introduction: Binge use of methamphetamine leads to intense euphoria and other stimulatory effects followed by dysphoria and rebound central nervous system (CNS) depressant effects during the crash and withdrawal stages. In addition to physiological effects such as motor restlessness, dilated pupils, and increased pulse and blood pressure, at lower doses users may experience increased focus. However, at recreational doses user may experience significant impairment including agitation, hyperreflexia, irritability, delusion, paranoia, and dissociative behaviors. Impairment cannot be determined solely from blood concentration due to a hysteresis loop between physiological effects and concentration. The sympathomimetic nature and varying adverse effects of methamphetamine during different use phases pose a significant risk to driving. Over the last six years, there has been a 7.4% increase in the screen positivity for methamphetamine/MDMA in the routine driving under the influence of drugs (DUID) panel at NMS Labs. This rise underscores the importance of re-examining methamphetamine concentrations, driving behaviors, and physiological profiles in drivers suspected of impairment solely due to methamphetamine, as determined by toxicological analysis.

Objectives: The aim of this research was to review toxicological findings and Drug Recognition Evaluation (DRE) reports for positive methamphetamine cases in the DUID population from 2018 through 2023 to show the relationship between observed impairment and methamphetamine positivity, regardless of concentration, and to document patterns of observed behaviors.

Methods: Blood samples were analyzed for the presence of methamphetamine via liquid chromatography tandem mass spectrometry (LC-MS/MS). Cases were evaluated to categorize polydrug use vs. methamphetamine-only cases. The detection of a parent drug and its metabolite were identified as one drug finding in this evaluation.

Eleven DRE reports were obtained from the Institute for Traffic Safety Management & Research (ITSMR) where methamphetamine/amphetamine was the only drug identified by NMS Labs through toxicological analysis of blood samples. These reports were evaluated for demographics, behaviors, vital signs, eye examination and standard field sobriety test results, and other physical indicators of impairment.

Results: Of the cases analyzed (n=16,866), methamphetamine concentrations ranged between 5-13000 ng/mL, with a mean of 366 ng/mL and a median of 250 ng/mL. Approximately 26% of the total population tested positive for methamphetamine <100 ng/mL. Amphetamine was detected in 91% of methamphetamine positive cases with concentrations ranging between 5-1600 ng/mL, with a mean of 48 ng/mL and a median of 35 ng/mL. In poly-drug cases (74% of the study population), more than half were positive for 3 or more drugs. Cannabinoids, fentanyl, cocaine, buprenorphine, and alprazolam were present most commonly along with methamphetamine.

In cases where methamphetamine was the only finding (n=4,147), methamphetamine concentrations ranged between 5-13000 ng/mL, with a mean of 468 ng/mL and a median of 350 ng/mL. Amphetamine was detected in 97% of methamphetamine positive cases with concentrations ranging between 5-460 ng/mL, with a mean of 52 ng/mL and median of 41 ng/mL.

The major findings from the 11 DRE reports were as follows: 82% of subjects exhibited increased average pulse (>90 beats per minute), all subjects showed dilated or normal pupil size. 72% exceeded the acceptable range (30±5 seconds) for the 30-second estimation test, and most subjects showed poor coordination. Additional findings will be discussed in the final poster presentation.

Conclusion/Discussion: Evaluating a DRE report in conjunction with the toxicological findings is crucial for understanding the nature of impairment observed at the time of the incident. Relying solely on methamphetamine concentrations from toxicological analysis to determine an individual's level of impairment without context is inappropriate. Both acute effects of high dose use and withdrawal effects from chronic use produce significant but distinct impairments, with the

profile of withdrawal effects often differing from the expected DRE matrix symptoms of stimulants.

P121

Z-Drug Concentrations in Postmortem Investigations, 2021-2023

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Introduction: The Z-drugs zolpidem, zaleplon, eszopiclone, and its stereoisomer zopiclone are non-benzodiazepine sedative-hypnotics used to treat insomnia. They bind to the same gamma-aminobutyric acid receptors as benzodiazepines but have a shorter duration of action and half-life, making them more clinically preferable for insomnia treatment. Z-drugs are considered to have relatively low toxicity at therapeutic doses for their indicated conditions. Deaths from Z-drugs are rare and more likely to occur with polydrug use or because of accidents caused by complex sleep behaviors.

Objectives: This presentation will explore Z-drugs and identify postmortem cases where concentrations were consistent with therapeutic, toxic, and lethal levels.

Methods: Postmortem blood specimens submitted to NMS Labs between January 2021 and December 2023 were evaluated for Z-drugs. Toxicological analyses were performed by directed analysis or as a screen with subsequent confirmation. The screening and confirmation reporting limits for the Z-drugs are in parentheses. Specimens were screened by Liquid Chromatography/Time of Flight/Mass Spectrometry (LC/TOF/MS) for all Z-drugs (10 ng/mL). Confirmation testing was performed using High-Performance Liquid Chromatography/Tandem Mass Spectrometry (LC-MS/MS) for zolpidem (1 ng/mL), eszopiclone/zopiclone (2 ng/mL), and zaleplon (4 ng/mL). Enantiomeric separation for eszopiclone/zopiclone was not performed.

Results: From 2021 to 2023, NMS Labs quantified Z-drugs in 4226 postmortem cases: Zolpidem (*n* = 2836), eszopiclone/zopiclone (*n* = 1364), and zaleplon (*n* = 44). Multiple Z-drugs were present in 24 cases. Z-drug blood concentrations were between subtherapeutic and potentially lethal levels. Whole blood concentrations ranged from 4.3 to 120000 ng/mL for zolpidem (median: 63 ng/mL, IQR [Q1-Q3]: 26-180), 2 to 20000 ng/mL for eszopiclone/zopiclone (median: 34 ng/mL, IQR: 13-100), and 4 to 3200 ng/mL for zaleplon (median: 42 ng/mL, IQR: 20-118).

Discussion: Zolpidem concentrations following therapeutic dosing are approximately 100 to 250 ng/mL in whole blood. Zolpidem has been implicated in polydrug deaths at concentrations between 1100 and 4500 ng/mL and greater than 4000 ng/mL in zolpidem-only fatalities. In this study, 19% of zolpidem-positive cases exceeded concentrations reported following therapeutic dosing, 4% had concentrations consistent with those observed in co-ingestion deaths, and 1% had concentrations associated with zolpidem-only fatalities. Whole blood concentrations are approximately 8 to 72 ng/mL for eszopiclone and 55 to 85 ng/mL for zopiclone after therapeutic doses. Both eszopiclone and zopiclone can exhibit toxicity at blood concentrations greater than or equal to 150 ng/mL. Nineteen percent of eszopiclone/zopiclone cases had concentrations above 150 ng/mL. There are no reports of fatalities in the literature attributed to eszopiclone alone or eszopiclone co-ingestion. Contrastingly, zopiclone deaths have occurred at concentrations above 600 ng/mL when only zopiclone was present and between 250 and 4000 ng/mL when co-ingested with other substances. Because enantiomeric differentiation was not performed, we could not determine how many cases met these criteria for zopiclone. Zaleplon concentrations after therapeutic dosing are 1 to 100 ng/mL in whole blood. Here, 27% of zaleplon-positive cases exceeded 100 ng/mL. Due to its short duration, adverse effects of zaleplon are less common, and fatalities solely attributed to zaleplon have not been reported. However, concentrations greater than 1000 ng/mL have been implicated in fatal polydrug overdoses. Two percent of zaleplon-positive cases were above this level.

While Z-drugs are effective at treating insomnia, they are not without adverse side effects, especially when taken with other CNS depressants. In this study, most Z-drug cases had concentrations consistent with therapeutic levels. Additionally, most cases were positive for other drugs that could be toxic in combination with Z-drugs, including other CNS depressants.

P122

Determining the detection of 215 Fentanyl Analogs and Synthetic Opioids using Four Commercial Immunoassays

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Introduction: The United States continues to be plagued by the opioid epidemic and the third wave predominated by fentanyl analogs has yet to abate. Detectability of fentanyl analogs and synthetic opioids is of high interest to clinical and forensic laboratories. The US Centers for Disease Control and Prevention (CDC) has coordinated production of a Traceable Opioid Material® Kits (TOM Kits®) to support laboratory detection of current and potentially emerging opioids as well as common co-drugs found in fentanyl-containing samples.

Objectives: Previously published studies by this group on the topic have utilized analysis of compound structure as related to assay detectability to extrapolate the likely target epitope, the final data analysis of these >1,800 measurements will also include this prediction.

Methods: From the TOM Kits®, 215 opioids were evaluated, which included 194 fentanyl analogs, 17 synthetic opioids, and 4 internal standards. The opioids were analyzed using four commercially available homogeneous fentanyl immunoassay screening kits at their respective manufacturer's cutoff concentration. The immunoassays used were alphabetically (Tradename – Manufacturer): ARK™ Fentanyl II (Ark Diagnostics), Fentanyl Urine HEIA® Drug Screening Kit and Fentanyl Urine SEFRIA® Drug Screening Kit (Immunalysis Inc.), and the LZI Fentanyl Enzyme Immunoassay (Lin-Zhi International, Inc.). The analysis was performed on an Abbott Architect Plus c4000 (Abbott Diagnostics). The detectability of the opioids was initially evaluated by preparing each opioid individually in certified drug-free human urine at 1 ng/mL, and then analyzing the sample using the four immunoassays to determine if the analog screened positive at the assay's cutoff in singlicate. If the result was positive, no further testing was performed for that opioid using that immunoassay. If the result was negative, the resultant value was evaluated to determine if a 10 or higher 100 ng/mL concentration was appropriate for the second analysis. Opioids not screening positive at 100 ng/mL were considered not detected by that immunoassay.

Results: The difference in reactivity of the immunoassay's reagents was evaluated in conjunction with the chemical structure of each opioid. All four immunoassays were able to detect 106 of the opioids at the concentrations tested. Eighty-two opioids had variable cross-reactivity with the four immunoassays determined by the unique epitope for each reagent antibody. All four immunoassays were not able to detect 28 opioids and 17 of these were the emerging synthetic opioids. At the lowest concentration tested (1 ng/mL), the ARK II assay detected 36 compounds, the SEFRIA detected 74, LZI detected 5, and the HEIA detected 18. Higher concentration testing was conducted on the other compounds. The undetected opioids included the surgical anesthetic parent compounds remifentanil and alfentanil, and sufentanil metabolite norsufentanil. Additionally, three carfentanil analogs were not detected by the four immunoassays. Of the 34 compounds only detected by one assay, 27 of those positive results were from the Lin-Zhi assay.

Discussion: This information will be of use in both clinical and forensic settings in evaluation of the potential false-positive or false negatives in urine fentanyl screening. The detectability of fentanyl analogs and synthetic opioids varies by assay and it may be possible to predict the detection or lack thereof for a particular assay based on a pattern elucidated by the analysis of the TOMs Opioid compound set. This data may be used to evaluate the potential false negative or false positive results of commercially available fentanyl and norfentanyl homogeneous immunoassays and to predict the likely target epitope of each assay.

P123

Design of Experiment Studies to Evaluate Extraction Parameters for the Detection of Chronic and Single Dose Drug Exposure in Forensic Hair Analysis

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Background/Introduction: Forensic toxicology gains numerous advantages from drug analysis of hair including evaluating exposures that extend beyond the typical detection windows of other specimens. It is a valuable matrix for identifying long-term and chronic drug use, but can also be helpful for detecting single dose exposures. To date, there has been limited research on single-dose hair analysis, remaining a major challenge in forensic toxicology. This can be especially helpful in cases of drug facilitated sexual assault (DFSA) and drug facilitated crime (DFC), where a single dose of a drug is suspected. As the expected concentration range of drugs in hair following a single dose exposure is in the low pg/mg range, highly sensitive and specific methods are required.

Objectives: Hair analysis procedures can be extremely tedious, requiring several steps and multiple changing variables. This creates the need for an optimized working method for forensic hair analysis, which is the overall goal of this research. This study employed a statistical Design of Experiment (DoE) approach to compare hair extraction parameters for drugs and metabolites using authentic hair reference materials (HRM). A primary aim was to optimize the extraction procedure and LC-QqQ-MS method to facilitate the analysis of single dose samples.

Methods: This project utilized a DoE protocol and evaluated different sample sizes and extraction times for the solvent swelling extraction technique. An ultrasonication step was introduced, which has been shown to improve recovery of certain drugs from hair. The previously optimized decontamination procedure consisted of one 30-min wash with water followed by three 30-min washes with dichloromethane. The hair was then dried overnight, weighed out, and placed into a steel milling jar. For homogenization, it was pulverized in a Mini-Bead Beater 24 ball mill (Biospec; Bartlesville, OK, USA) in 10 s intervals for 30 s at 3200 rpm, resulting in finely ground hair. The solvent swelling technique consists of incubating the hair in a mixture of methanol, acetonitrile, and 2 mM ammonium formate (25:25:50, v/v/v) at 37ºC. The project included a 2^3 factorial DoE assessment for different extraction parameters to optimize the method for detection of single doses of drugs in hair. The DoE design included factor levels as follows: Factor A – sample size, 5 mg (-) or 20 mg (+); Factor B - extraction time, 2 h (-) or 6 h (+); and Factor C – with (+) and without (-) ultrasonication.

The final LC-QqQ-MS method includes a total of 43 compounds that are potentially relevant to DFC cases, with deuterated internal standards for each analyte. The method used an Agilent 1290 UHPLC coupled to a 6470 LC-QqQ-MS/MS with electrospray ionization in positive and negative modes. The column used was a Zorbax Eclipse Plus C18 column 3.0 x 100 mm, 1.8 mm with guard column. Aqueous mobile phase (A) is 5 mM ammonium formate in 0.1% formic acid in water and organic phase (B) is 0.1% formic acid in methanol. The gradient starts at 5% B for 5 min then increases to 95% until 8 min and is held for 2 min at a flow rate of 0.3 mL/min. Calibration curves were matrix matched using blank hair with the majority of the LOD and LOQ values at ranges of .01-.26 pg/mg and .03-.81 pg/mg, respectively.

Conclusion: The LC-QqQ-MS method demonstrates good selectivity and sensitivity for all 43 drug compounds, suitable for the detection of low concentrations of drugs in hair consistent with single doses. Future work will include the validation of this optimized method using the ANSI/ASB Standard 036 guidelines and then application for the use of detecting single doses of drugs in hair using authentic single dose specimens.

P124

Characterization of Xylazine's Rise within San Francisco

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Introduction: Xylazine, a veterinary sedative, has recently migrated from the United States East Coast to the West Coast, and has become prevalent in the San Francisco drug market. It has commonly been used as an adulterant in the illicit fentanyl supply. Although the use of xylazine, which is typically unknowingly added into the illicit drug supply, has been observed of other parts of the United States, San Francisco has seen its introduction and rapid growth starting in early 2022. Reports of its detection throughout California and neighboring states is lacking.

Objectives: The objectives of this study were to determine the prevalence of xylazine in the forensic casework within San Francisco Office of the Chief Medical Examiner (SFOCME), identify trends in accidental overdoses over time, and categorize concurrent substance use with xylazine.

Methods: The SFOCME performs full and comprehensive toxicological testing of over 200 drug and metabolites routinely on all postmortem, driving under the influence of drugs and/or alcohol (DUID), drug facilitated sexual assault (DFSA) and publicly intoxicated (PI) casework. Inclusion criteria encompasses all cases with toxicology testing performed in 2023 and all accidental overdose cases from 2022.

Results: Among accidental overdose cases, xylazine prevalence rose to 11 cases in 2022, and increased four times to 48 cases in 2023. The mean concentration remained consistent from 27 ng/mL (median: 3.7 ng/mL, min: 0.45 ng/mL, max: 160 ng/mL) in 2022 to 26 ng/mL (median: 1.8 ng/mL, min: 0.01 ng/mL, max: 840 ng/mL) in 2023. In 2023, xylazine was also observed in 36 (41%) PI cases, 3 (1%) DFSA cases and 2 (<1%) DUID cases. Further, all cases with xylazine present, also showed the detection of fentanyl, a fentanyl precursor or metabolite, or a fentanyl analog. Moreover, all PI cases included multiple additional positive drug results.

Discussion: Xylazine was four times more prevalent among accidental overdose cases in 2023. Although the mean and median concentrations remain relatively similar there was a 5 times increase in the maximum concentration. The rapid increase in xylazine prevalence in combination with the exclusive use with a fentanyl related compound supports the notion that xylazine was recently introduced into the San Francisco illicit fentanyl market and is increasing in concentration in that supply as overtime. Further, using PI cases as a reflection of drug use in San Francisco, xylazine is actively being used with a variety of other impairing substances. This trend may be attributed to xylazine's use as a fentanyl adulterant combined with general polysubstance use or the adulteration of other drugs of abuse. While an increase in xylazine alone is noteworthy, its presence alongside a fentanyl compound is even more alarming due to synergistic toxic effects, and inability to be reversed by naloxone or other mu-opioid receptor antagonists. The continued rise in the prevalence and concentration of xylazine is expected to lead to more accidental overdose deaths and harm in other areas in the community.

P125

The Evaluation of "Hemp-derived" Cannabis Product Mislabeling Using LC-MS/MS

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Introduction: An overproduction of cannabidiol (CBD) resulted from the 2018 Agricultural Improvement Act, which federally legalized hemp and CBD. The excess CBD led to the production of synthetic and semi-synthetic cannabinoids, marketed as "legal" and "hemp-derived". Unlabeled and mislabeled compounds in products have resulted in untoward and unexpected adverse events. Since the unregulated cannabis industry is always evolving and producing a vast number of new compounds, laboratories have struggled to keep updated analytical methods.

Objectives: This study aimed to develop and validate a liquid chromatography-tandem mass spectrometry (LC-MS/MS) method for the separation and quantitation of 21 cannabinoids. Additionally, the study evaluated the mislabeling of commercially available "hemp-derived" cannabis products.

Methods: Separation was achieved using a Shim Pack Volex C18 3.0 x 150mm, 2.7 µm column held at 40ºC and a Shimadzu LCMS 8050. A binary gradient at 0.6 mL/min with 0.1% formic acid in water for mobile phase A and methanol for mobile phase B was utilized. Mobile phase B was held at 83% for 12.40 min and increased to 100% at 12.41 min. Mobile phase B was held at 100% from 12.41-15.50 min, then at 15.51 min mobile phase B was decreased to 83% and held for 2 min. The inter- and intra-precision and bias, carryover, linearity, limit of detection (LOD), limit of quantitation (LOQ), and post-preparative stability were evaluated. A seven-point calibration curve from 10 - 1000 ng/mL of Δ^9-tetrahydrocannabinol (THC), Δ^8-THC, Δ^{10}-THC, $\Delta^{6a,10a}$-THC, cannabichromene (CBC), cannabidivarin (CBDV), cannabidiolic acid (CBDA), cannabigerol (CBG), CBD, cannabinol (CBN), tetrahydrocannabinolic acid (THCA-A), hexahydrocannabinol (HHC), Δ^9-tetrahydrocannabutol (THCB), Δ^9-tetrahydrocannabiphorol (THCP), Δ^8-THCP, Δ^9-tetrahydrocannabihexol (THCH), CBD-di-acetate, CBN-acetate, Δ^9-THC-acetate, and HHC-acetate with deuterated internal standards of Δ^9-THC-d_3, CBD-d_3, Δ^9-THC-acetate-d_3, and CBD-di-acetate-d_3 was used. A total of 55 products labeled to contain cannabinoids were purchased and analyzed using the developed method. The products were evaluated for labeling and concentration accuracy.

Results: The method successfully separated and quantitated the phytocannabinoids, semi-synthetic, and synthetic cannabinoids in e-liquids and edibles. Analysis of the cannabis products illustrated the poor quality assurance and labeling practices of the unregulated market as 65.5% of products contained all the labeled cannabinoids but also contained additional unlabeled cannabinoids, and 10.9% of products did not contain all the labeled cannabinoids and may or may not have contained unlabeled cannabinoids. Additionally, many cannabinoids were present at extremely high concentrations and the labeled concentrations were often inaccurate.

Conclusion/Discussion: There is little pharmacological data available for most of these emerging synthetic and semi-synthetic cannabinoids, posing a risk to public health and safety. Consumers are increasingly reporting adverse events to poison controls centers after consuming cannabis products, however the cannabinoids involved are unknown. It is imperative to develop comprehensive methodologies to accurately define these adverse events and to ensure the public receive safer products.

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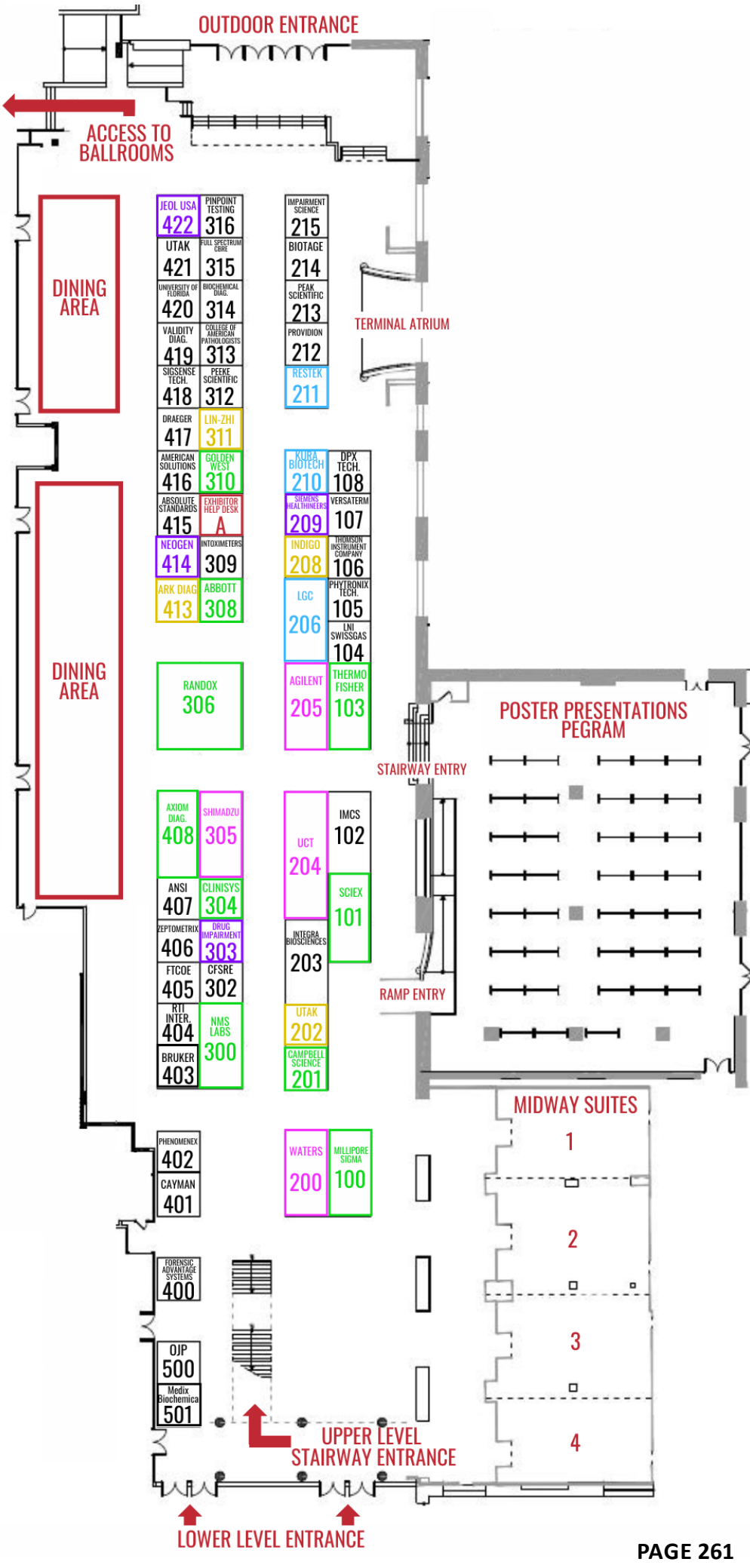

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At DPX Technologies, we believe that sample preparation should be fast and simple. We have developed a variety of sample preparation product lines in easy-to-use pipette tips. Our patented dispersive pipette eXTRaction (XTR) tip functions by dispersive SPE, requiring only seconds of mixing to complete each step of the sample preparation process. The patented Tip-on-Tip technology is used to filter samples by dispensing them through the DPX Filtration tip. The latest DPX product line, INTip Size Exclusion Chromatography, provides a unique, patent pending process for automated group separations for buffer exchange, desalting and PCR cleanup. Whether your laboratory uses a single channel pipettor or a fully robotic liquid handler, there is a DPX tip compatible with your analysis method and throughput. Stop by our booth to learn how DPX products can streamline your workflow.

EXHIBITORS

Draeger, Inc.
Booth 417
Sharon Roach
Sharon.roach@draeger.com
https://www.draeger.com/en-us_us/Safety/Law-Enforcement-Solutions

At Dräger, Inc., we are guided by the driving philosophy of "Technology for Life," which fuels our unwavering commitment to creating innovative solutions that protect the people who protect our nation. Our advanced evidential alcohol and drug testing technologies are specifically designed to meet the rigorous demands of law enforcement, ensuring that officers have the most reliable and precise tools available to maintain public safety and uphold the law with confidence and integrity.

One of our flagship products, the **Dräger DrugTest 5000**, is an oral fluid drug screener that provides a fast, reliable, and non-invasive method for detecting recent drug use. This innovative device screens for a wide range of substances, including marijuana, cocaine, opiates, amphetamines, methamphetamines, and benzodiazepines. The Dräger DrugTest 5000 is designed to deliver results in just a few minutes, allowing officers to make quick, informed decisions in the field. Its portability, ease of use, and accuracy make it an invaluable tool for roadside checks, accident investigations, and more.

In addition to drug screening, Dräger offers the **Alcotest 6000**, a robust and sturdy breath alcohol screener in alcohol detection. The Alcotest 6000 is built on decades of research and development, stemming from our roots of our first breath screener in 1956. Incorporating advanced sensor technology to provide highly accurate and reliable results this device is user-friendly, featuring an intuitive interface and quick, hygienic operational readiness, ensuring that officers can perform breath tests efficiently and effectively. The Alcotest 6000 is suitable for roadside screening and pre-checks making it a versatile tool for various law enforcement scenarios and workplace testing.

Dräger's innovative solutions are backed by comprehensive support and training programs. We understand that the effectiveness of our devices is not only determined by their technology but also by the proficiency of the users. Therefore, we offer extensive training sessions and resources to help law enforcement personnel be well-equipped to use our products to their full potential.

Moreover, our solutions can integrate seamlessly with existing law enforcement protocols and systems, ensuring that officers can use them without disrupting their workflow. The ease of integration and user-friendly design of our devices means that officers spend less time learning new technology and more time focusing on their critical duties.

Our mission at Dräger is to enhance public safety through technology and innovation. By providing law enforcement agencies with advanced drug and alcohol detection tools, we help officers perform their duties more effectively, reducing the risk of impaired driving and drug-related incidents. We are proud to support the brave men and women who serve our communities, and we remain dedicated to developing solutions that protect lives and promote justice.

EXHIBITORS

DrugImpairment.com
Booth 303
Tier V Sponsor
Matthew Myers, MS, MPA
Info@DrugImpairment.com
https://DrugImpairment.com

DrugImpairment.com connects toxicologists and law enforcement professionals with industry leading instructors and advanced education on drug impairment in a platform that's mindful of limited budgets and scheduling challenges. Courses are led by many of the most prominent names in the industry and delivered through an e-learning platform that allows users to learn at their own pace and convenience. Everything in our continually growing catalog is included with membership, so there are no per-course fees. Continuing education certificates are granted upon completion and widely accepted for CEU credits from different credentialing bodies, including ABFT. Group accounts are available, allowing local agencies or state programs to get training for their whole team and self-administer learner accounts and progress. DrugImpairment.com also offers supporting resources that are purpose-built for forensic practitioners to get the information they need about drugs from a credible source without having to scour the web. Learn more at https://DrugImpairment.com

Finden by Kura Biotech
Booth 210
Tier III Sponsor
1201 W Peachtree St NW Ste 2625 PMB 95887, Atlanta, Georgia
Jose Luis Callejas
j.callejas@kurabiotech.com
www.kurabiotech.com

There is a reason every test is critical, and critical tests call for the best enzymes.

Determined to overcome the most common problems in drug testing, **Finden® is at the cutting edge of toxicological enzymology.**

We are committed to continuously improving the available enzymatic tools, making sample preparation in drug testing simple, fast, precise, and consistent. We are enabling laboratories to ensure a more accurate and reliable result to the patients, while at the same time increasing throughput.

Our 3rd generation enzyme, **B-One®**, is a masterpiece, allowing extremely fast, pure, and convenient enzymatic hydrolysis at room temperature.

B-One® reaches over 90% recovery within 15 minutes at room temperature of even the most difficult and highly concentrated analytes to hydrolyze.

EXHIBITORS

Forensic Advantage Systems

Booth 400

Brent Smith

bsmith3@forensicadvantage.com

https://www.forensicadvantage.com/

For the past 30 years, we have helped forensic laboratories improve efficiency and work processes through automation. We take a commonsense approach to automating complex forensic science processes for crime labs and medical examiners. Please stop by booth 400 and learn about how our new innovations LIMS technology and batch processing can help improve your lab operations.

Forensic Technology Center of Excellence (FTCOE)

Booth 405

https://forensiccoe.org/

forensiccoe@rti.org

(866) 252-8415

Mailing Address: RTI International, 3040 East Cornwallis Road, Research Triangle Park, NC 27709, United States

The Forensic Technology Center of Excellence (FTCOE), led by RTI International, is supported through a Cooperative Agreement from the National Institute of Justice (NIJ), Office of Justice Programs, U.S. Department of Justice (awards 15PNIJ-21-GK-02192-MUMU, 2016-MU-BX-K110, and 2011-DN-BX-K564). The FTCOE supports the implementation of new forensic technology and best practices by end users and is dedicated to elevating the status of forensic science through advancing technology, sharing knowledge, and addressing challenges. The FTCOE bridges the gap between the scientific and justice communities.

Advancing Technology: The FTCOE manages the testing and evaluation of emerging technologies applicable to forensic science. By identifying and removing the potential barriers that often derail the implementation and acceptance of new and innovative technologies, the FTCOE places promising technical innovations in the hands of forward-thinking practitioners, stakeholders, and policy makers. This is achieved through technical evaluation, technical assistance, and guidance resources.

Sharing Knowledge: The FTCOE provides knowledge transfer and integration which strengthens the connection between forensic discovery and forensic process using a blended learning environment consisting of web-based presentations, workshops, specialized events and discussions panels.

Addressing Challenges: The FTCOE is an established leader in the expeditious transition of research into the hands of practitioners. We understand the user needs, and through our support of the NIJ's Research and Development (R&D) portfolio, we identify and employ the requirements necessary to drive the adoption of research and development outcomes. To learn more about how the FTCOE supports the NIJ's R&D portfolio and provides technology transition support.

Social Media:

Twitter: @ForensicCOE https://twitter.com/ForensicCOE

Facebook: @ForensicCOE https://www.facebook.com/ForensicCOE

LinkedIn: https://www.linkedin.com/company/forensic-technology-center-of-excellence/

EXHIBITORS

Full Spectrum Lab Services from CBRE

Booth 315

Caitlin Cricco (Marketing Director, Life Sciences)

caitlin.cricco@cbre.com

www.fullspectrumlabservices.com

CBRE, 200 Park Avenue, 18th floor, NY, NY 10166

Full Spectrum Lab Services from CBRE offers a professional suite of services to a variety of clients in the life sciences industry, including those in pharmaceuticals, biotech, medical devices, and genomics, as well as contract research organizations (CROs) and contract manufacturing organizations (CMOs). We also service R&D clients across a broad range of industries including Oil & Gas, Telecomm, Forensics, Government Municipalities, and Technology.

Our comprehensive range of services includes laboratory consulting, real estate services, instrumentation repair and maintenance, full asset management, and more—covering the entire spectrum of life sciences facilities. With expertise across various life sciences categories, CBRE is a leader in managing highly-regulated spaces within the industry.

IMCS

Booth 102

Claire Collins, Director of Sales

inquiries@imcstips.com

www.imcstips.com

Integrated Micro-Chromatography Systems, Inc. (IMCS) is a privately-held biotech company that designs, manufactures, and distributes biological reagents from nextgeneration 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 βglucuronidase 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.

EXHIBITORS

Impairment Science, Inc.
Booth 215
1035 Cambridge Street, Suite One, Cambridge, MA 02141
Chris Bensley
chris@impairmentscience.com
https://www.impairmentscience.com/

About Impairment Science, Inc.

Impairment Science, Inc. (ISI) applies neuroscience research to detect and measure cognitive and psychomotor impairment from any cause. With the company's DRUID app and data platform, employers can assess employees' fitness for duty from any mobile device. Backed by multiple independent, published, peer-reviewed scientific studies, DRUID is the only technology proven to detect impairment from cannabis. Leading organizations in construction, manufacturing, mining, public safety, and transportation rely on DRUID to expand their ability to perceive and correct risks posed by impaired employees.

Indigo BioAutomation
Booth 208
385 City Center Dr., Suite 200, Carmel, IN 46032
Dave Vissing, Director of Sales
dvissing@indigobio.com
https://www.indigobio.com/

Indigo BioAutomation integrates advanced computational science with domain-specific expertise to offer innovative software and services that improve the performance of clinical and forensic laboratories. Our flagship products, ASCENT® for GC/LC-MS and ARQ® for PCR enable labs to confidently accelerate results review, efficiently deploy tests and optimize operations, and bridge scientific advancements with real-world impact.

Registered as Class 1 Medical Devices, ASCENT and ARQ integrate with popular lab hardware and software to streamline and automate lab specific standard operating procedures (SOPs), extend knowledge across the lab, and foster team collaboration from anywhere. Trusted by leading labs, the SaaS software securely processes, on average, more than 150K samples - encompassing over 8 million individual test results – per day, helping Indigo BioAutomation customers drive consistency at scale, decrease result turnaround times and sample repeats, and improve staff engagement.

For labs looking to leverage their continuously growing test and operational data for learning and optimization, the data generated by ASCENT and ARQ are securely stored and organized for access anytime, anywhere. Our data warehouse, designed to manage large-scale data analysis and processing within a secure and scalable cloud platform, is available to customers interested in tracking and analyzing their lab data, test results, and operational status using popular analysis and visualization tools, including PowerBI and Tableau. **Stop by booth #208 to find out more. Indigo BioAutomation: Your lab, elevated.**

EXHIBITORS

INTEGRA Biosciences

Booth 203

22 Friars Drive, Hudson, NH 03051

orders-us@integra-biosciences.com

www.integra-biosciences.com

INTEGRA is a leading provider of high-quality laboratory tools and consumables for liquid handling and media preparation. We are committed to fulfilling the needs of laboratory professionals in research, diagnostics, and quality control within the life sciences industry.

INTEGRA's globally recognized products include our EVOLVE manual pipettes, VIAFLO and VOYAGER electronic pipettes, VIAFLO 96/384, MINI 96, ASSIST and ASSIST PLUS, WELLJET reagent dispensers, PIPETBOY serologic pipettors, VACUSAFE and VACUSIP aspiration systems, DOSE IT peristaltic pumps, and lastly our MEDIACLAVE and MEDIAJET media preparation equipment. Visit www.integra-biosciences.com to see our full line of innovative products.

Intoximeters

Booth 309

2081 Craig Rd Saint Louis MO 63146

Rankine Forrester, CEO

sales@intox.com

www.Intox.com

Intoximeters: Pioneers in Breath Alcohol Testing Since 1945 - Intoximeters has built a reputation on experience, service, and integrity. As global leaders in breath alcohol testing, we remain at the forefront of innovation. Our in-house manufacturing, development, sales, marketing, training, and supportive services ensure unmatched quality in the law enforcement and workplace safety marketplace. We manufacture law enforcement-grade, evidential breath alcohol analyzers to assist in DUI enforcement. Our instruments are designed for accuracy, durability, and operator safety, featuring the highest quality and longest-lasting fuel cell in the market. We offer a range of devices for law enforcement and workplace testing, including handheld, portable, and desktop evidential-grade instruments.

JEOL USA, Inc.

Booth 422

Tier V Sponsor

11 Dearborn Road | Peabody, MA 01960

Tel: +1.978.536.2308

www.jeolusa.com

JEOL USA Inc. is a leading global supplier of electron microscopes, ion beam instruments, mass spectrometers and NMR spectrometers. JEOL's mass spectrometer product line includes GC-MS and GC-MS/MS systems as well as the revolutionary AccuTOF-DART "ambient ionization toolbox". JEOL's msFineAnalysis AI qualitative analysis software provides powerful non-targeted screening and structure analysis tools for GC-MS with a searchable database of mass spectra for over 100 million chemical structures.

EXHIBITORS

LGC Clinical Diagnostics

Booth 206

37 Birch St., Milford, MA 01757

Colleen Gang

kova.support@lgcgroup.com

https://kovaintl.com/

Biochemical Diagnostics, Inc. is a leading manufacturer of controls for drugs of abuse and pregnancy testing. Their DETECTABUSE®, SALIVABUSE®, STAT-SKREEN® and PREGNANCY-SKREEN products are utilized in clinical laboratories worldwide. Kova and BCD's dedication to delivering high-quality products and over 40 years of expertise has positioned them as a leading player in the industry. Both are certified in accordance with ISO 13485:2016 and are qualified cGMP manufacturers. In July 2023, Kova International, Inc and Biochemical Diagnostics, Inc. became a part of LGC Clinical Diagnostics as a strategic approach to broaden LGC Clinical Diagnostics product and service offerings that support accurate and reliable diagnostic results.

Lin-Zhi International, Inc.
Booth 311

Sponsor Tier IV

Direct: 408-320-9211, Main: 408-970-8811x1

www.lin-zhi.com

customerservice@lin-zhi.com

Lin-Zhi International, Inc. (LZI) is focused on innovative and quality products for clinical diagnostics. We lead the industry in the development of challenging assays that are liquid and ready to use. These novel assays include our FDA 510(k) cleared 6-Acetylmorphine (6AM), Buprenorphine (BUP), Ecstasy

(MDMA), Fentanyl (FEN), Hydrocodone (HYD), Methamphetamine (MAMP), Methadone Metabolite

(EDDP), Oxycodone (OXY III), Tramadol (TRAM), and FDA cleared De Novo Carisoprodol Metabolite (SOMA) Enzyme Immunoassays (EIAs).

In addition, LZI offers "Forensic Use Only" urinalysis reagents including Ethyl Glucuronide (EtG III), SPICE I (JWH-018), SPICE II (UR-144/XLR-ll), and Ketamine (KET) EIAs.

We also provide a full panel of Homogeneous Oral Fluid Enzyme Immunoassay reagents including the FDA 510(k) cleared Oral Fluid 6-Acetylmorphine (OF 6AM), Amphetamine (OF AMP), Methamphetamine (OF MAMP), and THC (OF THC) EIAs, as well as "Forensic Use Only" Oral Fluid EIA reagents including Oral Fluid Cocaine Metabolite (OF COC), Ecstasy (OF MDMA), Methadone (OFMTD), Methadone Metabolite (OF EDDP), Opiate (OF OPI), and Oxycodone (OF OXY) EIAs.

We invite you to become our partners as we continue to develop cutting-edge technology in the world of clinical diagnostics.

EXHIBITORS

Medix Biochemica

Booth 501

10850 Metro Court, Maryland Heights MO 63043

Edward Dy Edward.Dy@MedixBiochemica.com

www.medixbiochemica.com

Medix Biochemica is a market leading in vitro diagnostics raw material supplier with Finnish roots and global branches. We produce and supply high-quality antibodies, antigens and other critical IVD raw materials to enable our IVD customers to manufacture diagnostic tests and supporting materials all around the world. Our expertise covers market segments ranging from immunoassay, clinical chemistry and molecular diagnostics and capabilities include biospecimen accruals and contract manufacturing. With the most comprehensive raw material portfolio in the IVD world, and a team made up of the best minds in the business, we're ready to shorten your time to market and build quality into every one of your tests. Across disciplines and disease areas, whatever you need, chances are we IVDo that.

MilliporeSigma

Booth 100

Tier II Sponsor

400 Summit Drive

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Tihana Tomas

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www.milliporesigma.com

MilliporeSigma is the U.S. and Canada life science business of Merck KGaA, Darmstadt, Germany. With over 19,000 employees and 72 manufacturing sites worldwide, MilliporeSigma's portfolio spans more than 300,000 products enabling scientific discovery. Extensive expertise in laboratory water purification, separations, and reagents enables MilliporeSigma to provide advancements in chromatography including HPLC, UHPLC, TLC, and HPTLC for basic, applied, and pharmaceutical research and manufacturing. Visit us: https://www.sigmaaldrich.com/

Neogen

Booth 414

Tier V Sponsor

944 Nandino Boulevard, Lexington, KY 40511

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859-254-1221

Neogen® has supplied the forensic toxicology and workplace drug testing industries for more than 30 years and has become a trusted partner for drug screening solutions. We offer a wide range of immunoassays to detect drugs of abuse, including designer drugs and emerging drugs. Our solutions ensure confidence in your testing and provide reliable results.

EXHIBITORS

Our comprehensive line of drug detection assays includes an extensive range of 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.

We provide expert solutions, expert support, and exceptional results. At Neogen we don't just meet your drug-testing needs, we exceed them. Our team of experts is available to address your needs and determine how we can best support you.

NMS Labs
Booth 300
Tier II Sponsor
200 Welsh Road, Horsham, PA 19044
Kacie Tross, Kacie.Tross@nmslabs.com
www.nmslabs.com

NMS Labs is an international forensic and clinical laboratory that is unsurpassed in its scope of toxicology and drug identification testing, accuracy of results, scientific expertise, and innovation. The state-of-the-art headquarters includes forensic, clinical and research facilities, a dedicated and secure crime laboratory, and is staffed by more than 400 highly trained professionals. NMS Labs is passionate about promoting public health and safety.

Office of Justice Programs (OJP)
Booth 500
999 N. Capitol Street, NE
Washington, DC 20531
Contact Name: N/A
askojp@ncjrs.gov
https://www.ojp.gov

The Office of Justice Programs is the largest grantmaking component of the Department of Justice and houses the Department's criminal and juvenile justice-related science, statistics, and programmatic agencies. OJP provides federal leadership, funding, training and technical assistance, research and statistics, and other critical resources to advance work that strengthens community safety, promotes civil rights and equity, increases access to justice, supports crime victims and individuals impacted by the justice system, and builds trust between law enforcement and communities.

EXHIBITORS

PEAK Scientific Inc

Booth 213

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Westford, MA 01886

USA

John Norling

discover@peakscientific.com

https://www.peakscientific.com/

Discover why an on-site gas generator from Peak Scientific is the practical and cost-effective alternative to pressurized gas cylinders in your lab.

With nearly 30 years' pioneering reliable gas generator technology, Peak Scientific develops market-leading nitrogen, hydrogen and zero air systems mainly for the fields of LC-MS and GC.

We provide our customers with unrivalled peace of mind and hassle-free gas generation solutions for their specific laboratory demands. This is backed up by our world-class technical support and ongoing service care throughout the generator's lifespan. With a rapid response and offices on every continent we deliver a local service on a global scale.

Peeke Scientific

Novato, CA

Christopher Peeke

chris@peekescientific.com

www.peekescientific.com

Peeke Scientific specializes in the following:

- Manufacturing/Distribution of HPLC/UHPLC columns for clinical LC/MS
- Manufacturing/distribution of parts/kits for LC instruments to keep solvent vapors containedand compliant with safety rules Distribution of miscellaneous chromatography consumables

EXHIBITORS

Randox Toxicology

Booth 306

Tier II Sponsor

515 Industrial Boulevard, Kearneysville, West Virginia, 25430

info@randoxtoxicology.com

www.randoxtoxicology.com

With over 40 years of experience in the diagnostic market, Randox Toxicology is dedicated to advancing forensic, clinical, and workplace toxicology. Placing a heavy focus on new product R&D has led to the development of technology at the forefront of advanced global diagnostics. A market leader in the development of new assays and technology in the field of toxicology, Randox aims to minimize laboratory workflow constraints whilst maximizing the scope of quality drug detection. In 2023 Randox created the world's first commercially available Xylazine ELISA for toxicology screening, available also on our biochip ToxPlex panel.

With the world's largest toxicology test menu, screening for over 600 drugs and drug metabolites, our range of versatile analyzers provides toxicology solutions for both high and low-throughput laboratories.

Evidence MultiSTAT

A fully automated drug screening immunoassay analyzer

The Evidence MultiSTAT delivers simultaneous, semi-quantitative detection of up to 29 classical, prescription, and synthetic drugs and drug metabolites from a single sample. Designed to work across a variety of matrices, our patented Biochip Array Technology provides a complete immunoassay profile. The MultiSTAT offers a fully automated 3-step process:

1. Apply the sample
2. Load it into the machine
3. Press start

With a throughput of up to 176 tests per hour, the MultiSTAT offers rapid and highly accurate results. Our easy-to-use software is controlled via a 15.6" touchscreen, where results are displayed and can be printed or exported to LIMS.

Evidence Investigator

A semi-automated, compact benchtop analyser

The Evidence Investigator is a cost effective and efficient solution when testing for drugs of abuse, providing laboratories with a highly sensitive immunoassay screen. Designed for fast and accurate batch analysis, the Evidence Investigator can deliver up to 2,376 test results in 70 minutes. With only a small sample volume of 7-25uL required, more sample is left for confirmatory testing. The Evidence Investigator also facilitates testing of multiple matrices including; blood, urine, oral fluid, hair, vitreous humor, meconium and tissue to accommodate any laboratory.

Evidence +

A fully automated batch immunoanalyzer

With the potential of up to 5,280 test results per hour, the Evidence+ analyser is uniquely designed for fast and accurate batch analysis. The Evidence + allows the operator to save frequently used worklists, reload them onto the system and apply them to different arrays with a few simple clicks, ensuring time to first result is firmly fixed at <45 minutes.

Extended quality control viewing allows results to be displayed on the system for up to 180 days, facilitating the operator with trend analysis for recalibration and control performance. Configuration of the system enables message notifications and alerts to be emailed to the user, speeding up the day-to-day running of the laboratory whilst increasing walk away time and productivity.

RTI International

Booth 404

Laboratory Quality Assurance and Standardization

Lawrance D. Mullen, D.H.Sc, M.S. & Cynthia Lewallen, M.S

lmullen@rti.org; clewallen@rti.org

https://www.rti.org/focus-area/laboratory-quality-assurance-standardization

https://www.rti.org/impact/national-laboratory-certification-program-nlcp

https://forensicrti.org/nlcp/drug-testing-matters/

https://forensicrti.org/reference-materials/

Providing quality assurance services for drug testing laboratories.

RTI's laboratory quality assurance and standardization team assesses, supports, and improves the quality of drug testing laboratories. We develop and manage proficiency testing programs and forensic laboratory inspections as well as the design, preparation, and distribution of reference materials and quality assurance samples for clinical and forensic drug testing laboratories.

A defined quality management system enables efficient and effective procedures that comply with established technical and scientific standards, as verified by independent auditing authorities. Proficiency testing is used globally within forensic drug testing laboratories to ensure quality and standardization in laboratory operations and data interpretation. Our experienced toxicologists, chemists, and technicians ensure that our products and services meet or exceed community needs. To date, we have produced over 1 million proficiency testing samples for our clients in support of their critical need to verify the accuracy and reliability of forensic laboratory testing.

Quality assurance products and services support three main quality objectives:

- Produce materials and operate programs that meet or exceed customer requirements This may be measured by customer feedback and continued business relationships
- Produce materials and operate programs that meet the requirements of International Standards and other regulatory requirements. This is often measured by internal and external audits as well as continued accreditation
- Effectively use program resources to maximize cost efficiency

Our quality assurance skills include:

- Production of toxicology proficiency or performance testing samples and operation of the PT program in accordance with ISO 17043
- Production of reference materials in urine, oral fluid, hair, and blood in facilities that are accredited to ISO 17034
- Standard setting for laboratory accreditation
- Support of the development, validation, and maintenance of drug testing standards

While our quality assurance capabilities may be applied in many ways, our projects are designed to ensure the protection and safety of employers, donors, laboratory staff, and the public through accurate drug testing programs which serve as

the gold standard in laboratory accreditation.

The value of the work provided by the programs and laboratories we support impacts the confidence of the public and the other interested parties in the reliability and accuracy of the results produced. We are committed to complying with the strictest laboratory protocols to maintain the trust of the forensic community in these critically important quality assurance programs.

SCIEX

Booth 101

Tier II Sponsor

500 Old Connecticut Path, Framingham, MA 01701

Holly Pagnotta

holly.mccall@sciex.com

www.sciex.com/applications/forensics-testing

SCIEX offers 50 years of expertise in LC-MS/MS technology combined with a comprehensive porfolio of preconfigured LC-MS/MS methods, libraries and software designed to push the limits of speed and sensitivity for both quantitative and qualitative analyses. Our Triple Quad™, QTRAP® and QTOF systems provide enhanced sensitivity and usability enabling rapid screening, identification, and quantitation of hundreds of the most challenging compounds in a single analysis with confidence. SCIEX provides the expertise to help a new lab get running smoothly, as well as first-of-its-kind personalized online learning programs. For research only.

Siemens Healthineers

Booth 209

Tier V Sponsor

Since 1972, Syva® EMIT has been the trusted brand in drug testing. Syva Emit® reagents are one of the most widely used and extensively validated reagents in the industry.

Siemens Healthineers integrates full-spectrum drug testing diagnostics for detection of drugs-of-abuse and therapeutic drug monitoring. With a comprehensive product portfolio, Siemens drug testing diagnostics is the single-source solution for all your drug testing needs.

EXHIBITORS

Sigsense Technologies, Inc.

Booth 418

Craig Kreutzberg

craig.kreutzberg@sigsensetech.com

https://sigsensetech.com

The Sigsense AI-powered IoT platform analyzes equipment and workflows to generate a real-time view of your lab's performance, identifying trends and highlighting opportunities to optimize usage, eliminate downtime, and maximize productivity.

The platform is powered by our unique AI that provides deep, granular insights into equipment, workflow, and lab-wide operations—that looks like everything from reporting KPI metrics and tracking specific instrument functions like injections, to detecting batch disruptions and monitoring equipment condition to drive pre-emptive maintenance.

Be it for a single LC-MS stack or multiple fleets of various equipment types, the Sigsense platform helps improve throughput, identify workflow gaps, uncover causes of unproductive time, and much more.

About us: Recently acquired by Agilent, Sigsense Technologies leverages AI to help laboratories optimize and evolve their operations. Our clients span clinical, academic, and analytical labs, and include 6 of the top 10 global pharmaceutical companies. From real-time performance analysis and actionable insights to proactive failure prediction and prevention, we strive to help customers get the most out of the equipment their labs rely on.

Thermo Fisher Scientific

Booth 103

Tier II Sponsor

Tanis Correa

cmdevents.na@thermofisher.com

www.thermofisher.com

Thermo Fisher Scientific is the world leader in serving science. Our mission is to enable our customers to make the world healthier, cleaner and safer. Through a broad and complementary offering, we help the worlds toxicology community solve complex analytical challenges and increase laboratory productivity.

Our unique solutions address the entire toxicology workflow, from sample collection, screening with drugs of abuse kits and analyzers through to analytical confirmation. Our mass spectrometry solutions with triple quadrupole and Thermo Scientific™ Orbitrap™ technologies deliver confident, high-quality results supported by extensive mass spectral libraries for improved identification confidence. Differentiated front-end solutions limit the need for sample preparation and LC separation prior to MS analysis, increasing overall throughput.

EXHIBITORS

Thomson Instrument Company

Booth 106

Sam Ellis

htslabs.com

Thomson sells innovative single-use Solutions At Work™, our mission is to provide technical expertise while partnering with our customers to deliver practical scientific innovations enabling scientific advancements in pharmaceutical, bio-tech, environmental/food, toxicology/forensics, and contract manufacturing industries.

UCT

Booth 204

Tier I Sponsor

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University of Florida College of Pharmacy Forensic Science

Booth 420

Nancy Toffolo

https://forensicscience.ufl.edu/

The University of Florida College of Pharmacy Forensic Science Online Graduate Program:

Leading the Future of Forensics

Approaching its 25th anniversary, the University of Florida's Forensic Science Online Graduate Program is the largest and most prestigious program of its kind globally, shaping the next generation of leaders across four specialized concentration areas: Forensic Science, Forensic Toxicology, Forensic DNA and Serology, and Forensic Drug Chemistry. Most of our programs are offered through the College of Pharmacy, lauded as #4 nationally; this preeminent program provides comprehensive forensic education tailored for working professionals.

Leading-Edge Curriculum and Flexible Learning

The program's comprehensive online curriculum covers a wide range of forensic science topics. These specialized topics

include crime scene investigation, forensic toxicology, and forensic DNA analysis. Students have the flexibility to customize their studies by choosing from various elective courses, allowing them to align their education with their specific career goals. Through innovative online learning platforms and interactive virtual classrooms, students can engage with course materials, participate in discussions, and collaborate with peers and instructors from around the world, all while maintaining their professional and personal commitments. With rolling admissions and classes available year-round, students can pursue a Master's degree or graduate certificate or explore non-degree options - all without requiring GRE scores.

A World-Class Program with Award-Winning Faculty

The Forensic Science Online Graduate Program's excellence has been widely recognized, earning the prestigious Award of Excellence in Distance Education from the American Distance Education Consortium (ADEC). Additionally, the program's founder, Ian Tebbett, Ph.D., has received numerous accolades, including the Irving Award from ADEC and the Outstanding Leadership Award from the U.S. Distance Learning Association (USDLA).

Advancing Forensic Science Research and Practice

The University of Florida's Forensic Science Online Graduate Program is at the forefront of forensic science research and practice. Faculty members actively contribute to the field through groundbreaking research projects, publications, and consultations with law enforcement agencies and legal professionals. This real-world expertise ensures that students receive up-to date and industry-relevant knowledge, preparing them to tackle the complex challenges of modern forensic casework.

Join the Next Generation of Forensic Science Leaders

More than 1,500 alumni of the Forensic Science Online Graduate Program continue to set the standard for forensic education worldwide. Graduates of this program are equipped with the knowledge, skills, and credentials to advance their careers and contribute to the ongoing advancement of forensic science, making them sought-after professionals in various industries, including law enforcement, government agencies, and private forensic laboratories.

We invite you to join the next generation of forensic science leaders at the University of Florida. Apply today!

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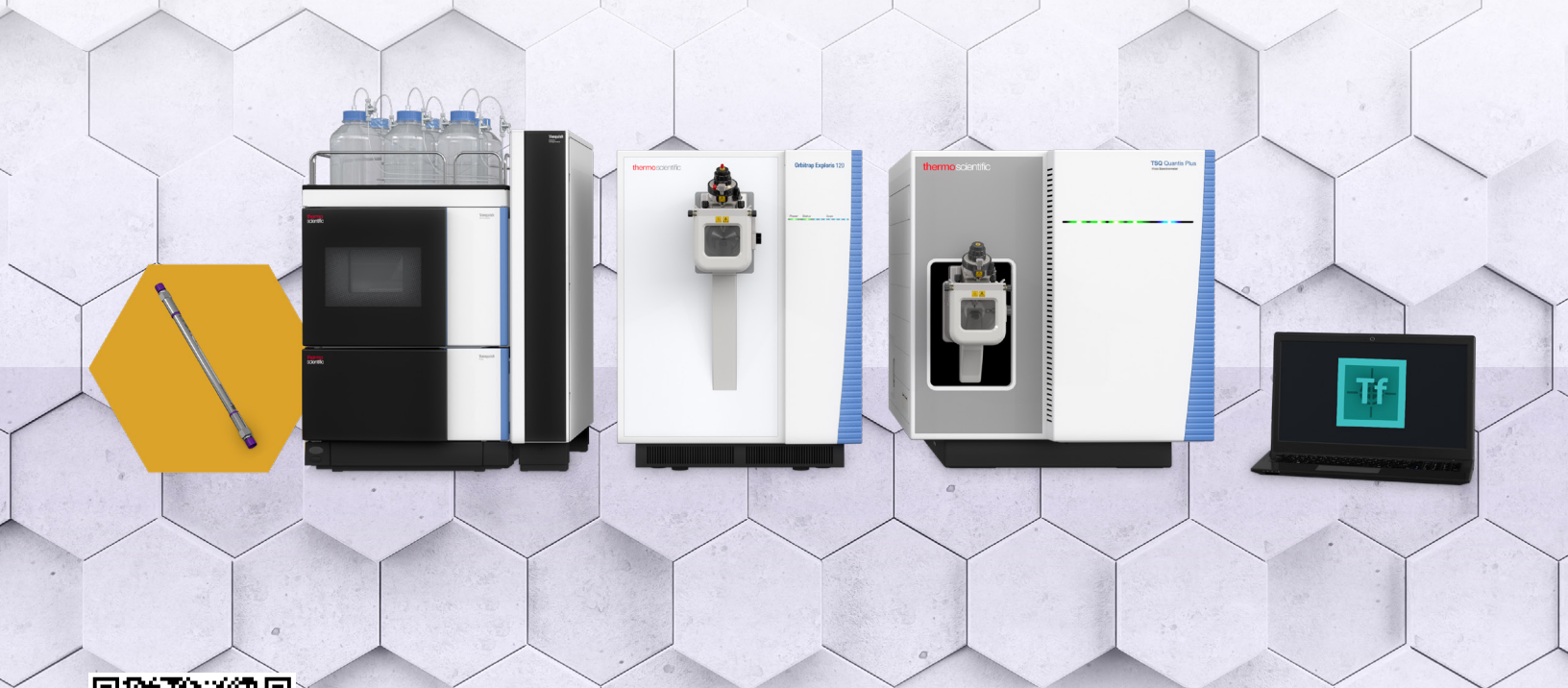

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Buprenorphine (5 & 10)	Methadone Metabolite (100 & 300)	Tramadol
Cocaine Metabolite (150 & 300)	Methamphetamine	
Cotinine II	Opiate (300 & 2000)	
Ecstasy	Oxycodone III (100 & 300)	
Ethyl Alcohol	Phencyclidine	
Ethyl Glucuronide III** (500 & 1000)	Propoxphene	

ORAL FLUID Drugs of Abuse Panel:

*All Products are FDA 510(k) Cleared Except Annotated * = De Novo Assay ** = Forensic Use*

6-Acetylmorphine	Ecstasy**	Oxycodone**
Amphetamine	Ethyl Alcohol**	Phencyclidine**
Barbiturate**	Methadone**	Propoxyphene**
Benzodiazepine**	Methamphetamine	THC**
Cocaine Metabolite**	Opiates**	

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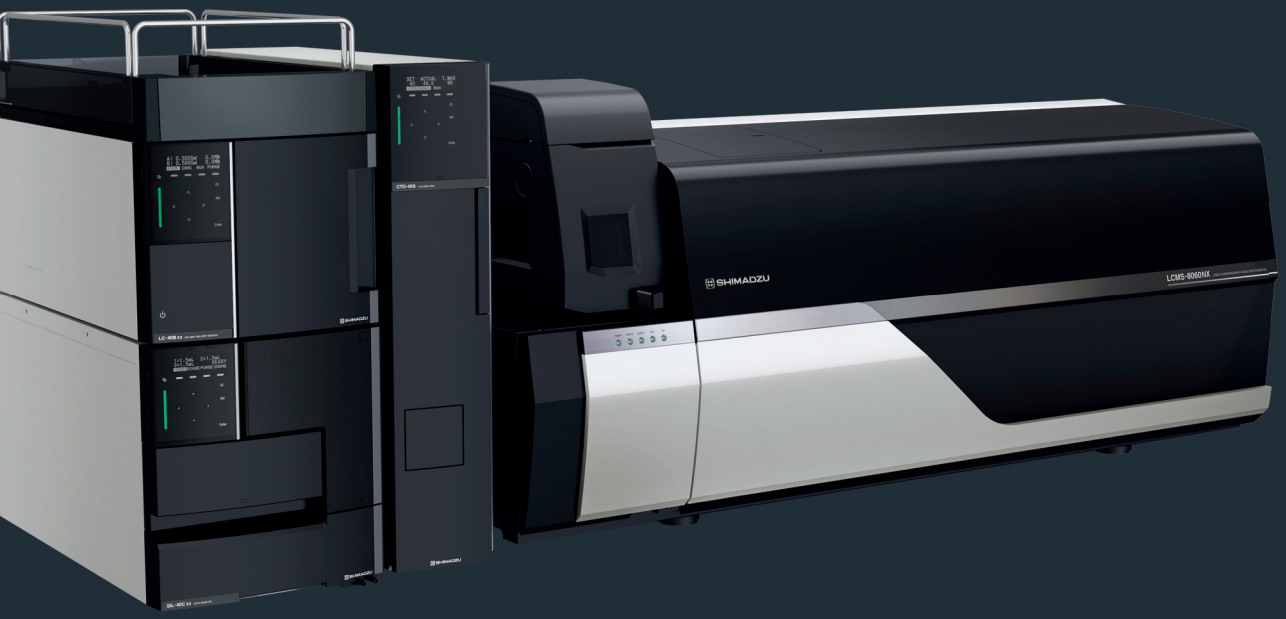

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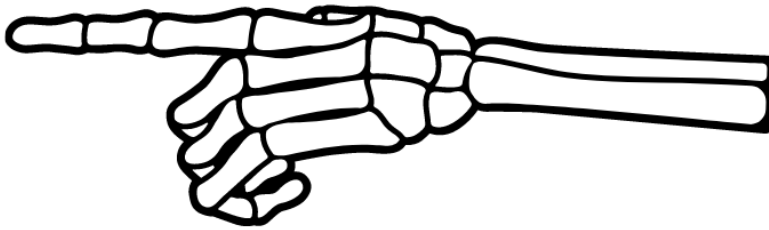

THANK YOU!

Thank you for joining us at SOFT 2024 in St. Louis! We hope you had a fantastic experience and enjoyed everything this year's meeting had to offer. We look forward to welcoming you to future SOFT meetings!

We are thrilled to invite you to SOFT 2025, which will be held at the **Oregon Convention Center from October 26 to October 31, 2025**. This exciting meeting will be **hosted by Sara Short and Amy Miles**, bringing together experts, practitioners, and enthusiasts in the field for a week of insightful sessions, networking opportunities, and engaging discussions. See you in Portland!

PLANNING COMMITTEE

Hosts: Sara Short & Amy Miles

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Workshop Program Coordinators: MaryLynn Heffington & Dani Mata

Food & Beverage Coordinators: Ann Marie Gordon, Denice Teem, Delisa Downey

Mobile Application Coordinators: Rusty Lewis, Roxane Ritter, Sunday Hickerson

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Fun Run Coordinators: Shannon Palladino

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JAT Special Issue Editor: To be announced at SOFT 2024!

Join us in the vibrant city of Chicago for the highly anticipated **SOFT/TIAFT 2026 Joint Meeting, hosted by Luke Rodda and Andre Sukta!** From **September 23 to October 2, the Hilton Chicago** will be the setting for an extraordinary gathering of toxicologists from around the world. Attendees will have the opportunity to exchange cutting-edge research, explore advancements in forensic science, and foster global collaborations. Set against the backdrop of the **iconic Windy City**, the event promises world-class scientific sessions, networking opportunities, and unforgettable social events. Don't miss this perfect blend of innovation, education, and Chicago's unique culture!

PLANNING COMMITTEE

Scientific Program Coordinators: Suman Rana, Donna Coy, Jennifer Schumann, Brigitte Desharnais

Workshop Program Coordinators: Craig Chatterton, Sue Pearring, Karen Scott

Food & Beverage Coordinators: Ann Marie Gordon, Denice Teem, Delisa Downey

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