

Hello to the SOFT Family,

Whether you are a long-time member or first-time attendee, you are part of the forensic toxicology family. We welcome you to SOFT 2022! It has been a long journey to bring SOFT to Cleveland and we are excited to host in this iconic Midwest city. We hope you enjoy your time here.

The planning committee has been working hard to organize a week full of learning opportunities. Monday and Tuesday workshops will address topics including process improvements, fentanyl, standard addition, new drugs, drugs and behavior, postmortem alcohol, method optimization, NPS benzodiazepines, testimony, and public health labs. Wednesday morning our plenary speaker, Jolene DeFiore-Hyrmer, MPH of the Ohio Department of Health, will present a talk on Unintentional Drug Overdose in Ohio: Using Population-based Data Sources and Surveillance with Toxicology Data to Drive Public Health Action. The scientific sessions begin on Wednesday and continue until Friday's closing ceremony. Be sure to visit the Exhibit Hall to view the poster sessions and meet with our Exhibitors. Some of our vendors are also providing lunch and learn opportunities early in the week, so don't miss out!

If you miss socializing with your colleagues or want to welcome new friends, there are a number of meet and greet opportunities. The events start as early as Sunday evening with the Young Forensic Toxicologists Symposium and continue throughout the week. On Tuesday, there is the Welcome Reception, Elmer Gordon Forum and MilliporeSigma Nite Owl Reception. Wednesday is the off-site event at the Rock and Roll Hall of Fame and Museum. At the Rock Hall, enjoy viewing the costumes, instruments and videos of a multitude of artists. Be sure to visit the New Inductee exhibit and Inductee signature gallery as well as enjoy food and drinks while listening to The Sunrise Jones, a talented five-piece cover band. The Karla Moore Fun Run will start bright and early on Thursday morning, so bring your walking or running shoes! Thursday night will be a night to remember as the SOFT family recognizes President Robert Sears at the "Red Carpet" President's Banquet. Gather at 6:00 pm for Happy Hour and an opportunity to take your picture on the red carpet. Dinner will be served at 7:00 pm followed by dancing to The Avenue, an energetic 10-piece band.

The SOFT 2022 planning committee has been an incredible group. Thank you to each of you for working together so well to deliver a successful meeting. Your talent and commitment were invaluable! A special thank you to the SOFT office, Beth and CC. Your knowledge and expertise in dealing with hotels, contracts, schedules, etc. was amazing. We appreciate how quick and thorough you were to answering our questions and providing guidance.

We look forward to welcoming all of you to Cleveland and hope you will enjoy the meeting and everything it has to offer. Take the opportunity to learn something new, meet with colleagues, visit with exhibitors, and have some fun!

-Doug and Shelly



2022 CO-HOST Shelly Merves Crosby, Ph.D., F-ABFT



2022 CO-HOST Doug Rohde, MS

Sunday, October 30, 2022	Time	Place
NIJ TWG Meeting	9am-5nm	Hilton - Veterans A
Registration	10am-5nm	Concourse
NSC-ADID Meeting	10am-1nm	Boom 06
NI CP Inspector Training	2-6nm	Room 01
YET Symposium	4:30-9pm	Atrium Ballroom
in roympoordin	1.00 0pm	Andin Banoom
Monday, October 31, 2022	Time	Place
Breakfast (WS Participants Only)	7-8:30am	Atrium
Registration	7am-6pm	Concourse
Student Enrichment Program	8am-5pm	Room 06
ABFT Lab Accreditation Committee	8am-5pm	Room 08
WS 2: Process Improvements	8am-5:30pm	Atrium Ballroom B
WS 3: Fentanyl	8am-5:30pm	Atrium Ballroom A
Break (WS Participants Only)	10-10:30am	Atrium
Abbott Lunch and Learn	12-1:30pm	Room 14
Agilent Lunch and Learn	12-1:30pm	Room 13
Sciex Lunch and Learn	12-1:30pm	Room 12
Thermo Lunch and Learn	12-1:30pm	Room 15
ABFT Exam Committee	1- 5pm	Room 03
WS 4: Standard Addition	1:30-5:30pm	Room 01
Break (WS Participants Only)	3:30-4pm	Atrium
NPS Committee	5:30-6:30pm	Room 03
SOFT-AAFS Drugs & Driving	5:30-6:30pm	Room 08
Thermo Fisher Reception	7:30-10:00pm	Atrium
		-
luesday, November 1, 2022	Time	Place
Iuesday, November 1, 2022 Breakfast (WS Participants Only)	Time 7-8:30am	Place Atrium
Iuesday, November 1, 2022 Breakfast (WS Participants Only) SOFT Board Meeting	Time 7-8:30am 7am-12pm	Place Atrium Room 06
Iuesday, November 1, 2022 Breakfast (WS Participants Only) SOFT Board Meeting Registration	Time 7-8:30am 7am-12pm 7am-6pm	Place Atrium Room 06 Concourse
Breakfast (WS Participants Only) SOFT Board Meeting Registration WS 5: New Drugs	Time 7-8:30am 7am-12pm 7am-6pm 8am-12pm	Place Atrium Room 06 Concourse Atrium Ballroom B
Iuesday, November 1, 2022 Breakfast (WS Participants Only) SOFT Board Meeting Registration WS 5: New Drugs WS 6: Drugs and Behavior	Time 7-8:30am 7am-12pm 7am-6pm 8am-12pm 8am-12pm	Place Atrium Room 06 Concourse Atrium Ballroom B Atrium Ballroom A
Breakfast (WS Participants Only) SOFT Board Meeting Registration WS 5: New Drugs WS 6: Drugs and Behavior WS 7: Postmortem Alcohols	Time 7-8:30am 7am-12pm 7am-6pm 8am-12pm 8am-12pm 8am-12pm	Place Atrium Room 06 Concourse Atrium Ballroom B Atrium Ballroom A Room 01
Iuesday, November 1, 2022 Breakfast (WS Participants Only) SOFT Board Meeting Registration WS 5: New Drugs WS 6: Drugs and Behavior WS 7: Postmortem Alcohols ABFT Exam	Time 7-8:30am 7am-12pm 7am-6pm 8am-12pm 8am-12pm 8am-12pm 8am-12pm	Place Atrium Room 06 Concourse Atrium Ballroom B Atrium Ballroom A Room 01 Room 03
Iuesday, November 1, 2022 Breakfast (WS Participants Only) SOFT Board Meeting Registration WS 5: New Drugs WS 6: Drugs and Behavior WS 7: Postmortem Alcohols ABFT Exam Break (WS Participants Only)	Time 7-8:30am 7am-12pm 7am-6pm 8am-12pm 8am-12pm 8am-12pm 8am-12pm 10-10:30am	Place Atrium Room 06 Concourse Atrium Ballroom B Atrium Ballroom A Room 01 Room 03 Atrium
Iuesday, November 1, 2022 Breakfast (WS Participants Only) SOFT Board Meeting Registration WS 5: New Drugs WS 6: Drugs and Behavior WS 7: Postmortem Alcohols ABFT Exam Break (WS Participants Only) Abbott Lunch and Learn	Time 7-8:30am 7am-12pm 7am-6pm 8am-12pm 8am-12pm 8am-12pm 8am-12pm 10-10:30am 12-1:30pm	Place Atrium Room 06 Concourse Atrium Ballroom B Atrium Ballroom A Room 01 Room 03 Atrium Room 14
Iuesday, November 1, 2022 Breakfast (WS Participants Only) SOFT Board Meeting Registration WS 5: New Drugs WS 6: Drugs and Behavior WS 7: Postmortem Alcohols ABFT Exam Break (WS Participants Only) Abbott Lunch and Learn Agilent Lunch and Learn	Time 7-8:30am 7am-12pm 7am-6pm 8am-12pm 8am-12pm 8am-12pm 8am-12pm 10-10:30am 12-1:30pm 12-1:30pm	Place Atrium Room 06 Concourse Atrium Ballroom B Atrium Ballroom A Room 01 Room 03 Atrium Room 14 Room 13
Iuesday, November 1, 2022 Breakfast (WS Participants Only) SOFT Board Meeting Registration WS 5: New Drugs WS 6: Drugs and Behavior WS 7: Postmortem Alcohols ABFT Exam Break (WS Participants Only) Abbott Lunch and Learn Agilent Lunch and Learn Bruker Lunch and Learn	Time 7-8:30am 7am-12pm 7am-6pm 8am-12pm 8am-12pm 8am-12pm 8am-12pm 10-10:30am 12-1:30pm 12-1:30pm 12-1:30pm	Place Atrium Room 06 Concourse Atrium Ballroom B Atrium Ballroom A Room 01 Room 03 Atrium Room 14 Room 13 Room 15
Iuesday, November 1, 2022Breakfast (WS Participants Only)SOFT Board MeetingRegistrationWS 5: New DrugsWS 6: Drugs and BehaviorWS 7: Postmortem AlcoholsABFT ExamBreak (WS Participants Only)Abbott Lunch and LearnAgilent Lunch and LearnBruker Lunch and LearnRandox Lunch and Learn	Time 7-8:30am 7am-12pm 7am-6pm 8am-12pm 8am-12pm 8am-12pm 8am-12pm 10-10:30am 12-1:30pm 12-1:30pm 12-1:30pm 12-1:30pm	Place Atrium Room 06 Concourse Atrium Ballroom B Atrium Ballroom A Room 01 Room 03 Atrium Room 13 Room 15 Room 9
Iuesday, November 1, 2022 Breakfast (WS Participants Only) SOFT Board Meeting Registration WS 5: New Drugs WS 6: Drugs and Behavior WS 7: Postmortem Alcohols ABFT Exam Break (WS Participants Only) Abbott Lunch and Learn Agilent Lunch and Learn Bruker Lunch and Learn Randox Lunch and Learn	Time 7-8:30am 7am-12pm 7am-6pm 8am-12pm 8am-12pm 8am-12pm 10-10:30am 12-1:30pm 12-1:30pm 12-1:30pm 12-1:30pm 12-1:30pm 12-1:30pm	Place Atrium Room 06 Concourse Atrium Ballroom B Atrium Ballroom A Room 01 Room 03 Atrium Room 14 Room 14 Room 13 Room 15 Room 9 Room 12
Iuesday, November 1, 2022 Breakfast (WS Participants Only) SOFT Board Meeting Registration WS 5: New Drugs WS 6: Drugs and Behavior WS 7: Postmortem Alcohols ABFT Exam Break (WS Participants Only) Abbott Lunch and Learn Agilent Lunch and Learn Bruker Lunch and Learn Randox Lunch and Learn Sciex Lunch and Learn	Time 7-8:30am 7am-12pm 7am-6pm 8am-12pm 8am-12pm 8am-12pm 8am-12pm 10-10:30am 12-1:30pm 12-1:30pm 12-1:30pm 12-1:30pm 12-1:30pm 12-1:30pm 12-1:30pm	Place Atrium Room 06 Concourse Atrium Ballroom B Atrium Ballroom A Room 01 Room 03 Atrium Room 14 Room 14 Room 13 Room 15 Room 9 Room 12 Room 10
Iuesday, November 1, 2022 Breakfast (WS Participants Only) SOFT Board Meeting Registration WS 5: New Drugs WS 6: Drugs and Behavior WS 7: Postmortem Alcohols ABFT Exam Break (WS Participants Only) Abbott Lunch and Learn Bruker Lunch and Learn Randox Lunch and Learn Sciex Lunch and Learn Shimadzu Lunch and Learn UCT Lunch and Learn	Time 7-8:30am 7am-12pm 7am-6pm 8am-12pm 8am-12pm 8am-12pm 8am-12pm 10-10:30am 12-1:30pm 12-1:30pm 12-1:30pm 12-1:30pm 12-1:30pm 12-1:30pm 12-1:30pm 12-1:30pm	Place Atrium Room 06 Concourse Atrium Ballroom B Atrium Ballroom A Room 01 Room 03 Atrium Room 14 Room 14 Room 13 Room 15 Room 9 Room 12 Room 10 Room 11
Iuesday, November 1, 2022 Breakfast (WS Participants Only) SOFT Board Meeting Registration WS 5: New Drugs WS 6: Drugs and Behavior WS 7: Postmortem Alcohols ABFT Exam Break (WS Participants Only) Abbott Lunch and Learn Bruker Lunch and Learn Bruker Lunch and Learn Randox Lunch and Learn Sciex Lunch and Learn Shimadzu Lunch and Learn UCT Lunch and Learn Waters Lunch and Learn	Time 7-8:30am 7am-12pm 7am-6pm 8am-12pm 8am-12pm 8am-12pm 10-10:30am 12-1:30pm 12-1:30pm 12-1:30pm 12-1:30pm 12-1:30pm 12-1:30pm 12-1:30pm 12-1:30pm 12-1:30pm	Place Atrium Room 06 Concourse Atrium Ballroom B Atrium Ballroom A Room 01 Room 03 Atrium Room 14 Room 14 Room 13 Room 15 Room 15 Room 9 Room 12 Room 10 Room 11 Room 11 Room 16
Iuesday, November 1, 2022 Breakfast (WS Participants Only) SOFT Board Meeting Registration WS 5: New Drugs WS 6: Drugs and Behavior WS 7: Postmortem Alcohols ABFT Exam Break (WS Participants Only) Abbott Lunch and Learn Agilent Lunch and Learn Bruker Lunch and Learn Randox Lunch and Learn Sciex Lunch and Learn Shimadzu Lunch and Learn UCT Lunch and Learn Waters Lunch and Learn ABFT Board Meeting	Time 7-8:30am 7am-12pm 7am-6pm 8am-12pm 8am-12pm 8am-12pm 8am-12pm 10-10:30am 12-1:30pm 12-1:30pm 12-1:30pm 12-1:30pm 12-1:30pm 12-1:30pm 12-1:30pm 12-1:30pm 12-1:30pm 12-1:30pm 12-1:30pm 12-1:30pm 12-5pm	Place Atrium Room 06 Concourse Atrium Ballroom B Atrium Ballroom A Room 01 Room 03 Atrium Room 14 Room 14 Room 13 Room 15 Room 9 Room 12 Room 12 Room 10 Room 11 Room 16 Room 08
Iuesday, November 1, 2022 Breakfast (WS Participants Only) SOFT Board Meeting Registration WS 5: New Drugs WS 6: Drugs and Behavior WS 7: Postmortem Alcohols ABFT Exam Break (WS Participants Only) Abbott Lunch and Learn Agilent Lunch and Learn Bruker Lunch and Learn Randox Lunch and Learn Sciex Lunch and Learn Shimadzu Lunch and Learn UCT Lunch and Learn Waters Lunch and Learn ABFT Board Meeting ConEd Committee Meeting	Time 7-8:30am 7am-12pm 7am-6pm 8am-12pm 8am-12pm 8am-12pm 8am-12pm 10-10:30am 12-1:30pm 1	Place Atrium Room 06 Concourse Atrium Ballroom B Atrium Ballroom A Room 01 Room 03 Atrium Room 14 Room 14 Room 13 Room 15 Room 15 Room 9 Room 12 Room 10 Room 11 Room 16 Room 08 Room 06
Iuesday, November 1, 2022 Breakfast (WS Participants Only) SOFT Board Meeting Registration WS 5: New Drugs WS 6: Drugs and Behavior WS 7: Postmortem Alcohols ABFT Exam Break (WS Participants Only) Abbott Lunch and Learn Agilent Lunch and Learn Bruker Lunch and Learn Randox Lunch and Learn Sciex Lunch and Learn Shimadzu Lunch and Learn UCT Lunch and Learn Waters Lunch and Learn Waters Lunch and Learn ABFT Board Meeting ConEd Committee Meeting WS 8: Method Optimization	Time 7-8:30am 7am-12pm 7am-6pm 8am-12pm 8am-12pm 8am-12pm 8am-12pm 10-10:30am 12-1:30pm 12-5pm	Place Atrium Room 06 Concourse Atrium Ballroom B Atrium Ballroom A Room 01 Room 03 Atrium Room 14 Room 14 Room 14 Room 15 Room 15 Room 9 Room 12 Room 10 Room 11 Room 11 Room 16 Room 08 Room 06 Atrium Ballroom B
Iuesday, November 1, 2022 Breakfast (WS Participants Only) SOFT Board Meeting Registration WS 5: New Drugs WS 6: Drugs and Behavior WS 7: Postmortem Alcohols ABFT Exam Break (WS Participants Only) Abbott Lunch and Learn Agilent Lunch and Learn Bruker Lunch and Learn Randox Lunch and Learn Sciex Lunch and Learn Sciex Lunch and Learn UCT Lunch and Learn Waters Lunch and Learn ABFT Board Meeting ConEd Committee Meeting WS 8: Method Optimization WS 9: NPS Benzos	Time 7-8:30am 7am-12pm 7am-6pm 8am-12pm 8am-12pm 8am-12pm 8am-12pm 10-10:30am 12-1:30pm 1	Place Atrium Room 06 Concourse Atrium Ballroom B Atrium Ballroom A Room 01 Room 03 Atrium Room 14 Room 14 Room 13 Room 15 Room 15 Room 9 Room 12 Room 12 Room 10 Room 11 Room 11 Room 16 Room 06 Atrium Ballroom B Atrium Ballroom A
Iuesday, November 1, 2022 Breakfast (WS Participants Only) SOFT Board Meeting Registration WS 5: New Drugs WS 6: Drugs and Behavior WS 7: Postmortem Alcohols ABFT Exam Break (WS Participants Only) Abbott Lunch and Learn Agilent Lunch and Learn Bruker Lunch and Learn Randox Lunch and Learn Sciex Lunch and Learn Sciex Lunch and Learn UCT Lunch and Learn Waters Lunch and Learn ABFT Board Meeting ConEd Committee Meeting WS 8: Method Optimization WS 9: NPS Benzos WS 10: Testimony	Time 7-8:30am 7am-12pm 7am-6pm 8am-12pm 8am-12pm 8am-12pm 8am-12pm 10-10:30am 12-1:30pm 1:30-5:30pm 1:30-5:30pm	Place Atrium Room 06 Concourse Atrium Ballroom B Atrium Ballroom A Room 01 Room 03 Atrium Room 14 Room 14 Room 13 Room 15 Room 15 Room 9 Room 15 Room 9 Room 12 Room 10 Room 11 Room 10 Room 11 Room 16 Room 08 Room 08 Room 06 Atrium Ballroom B Atrium Ballroom A Room 01
Iuesday, November 1, 2022 Breakfast (WS Participants Only) SOFT Board Meeting Registration WS 5: New Drugs WS 6: Drugs and Behavior WS 7: Postmortem Alcohols ABFT Exam Break (WS Participants Only) Abbott Lunch and Learn Agilent Lunch and Learn Bruker Lunch and Learn Randox Lunch and Learn Sciex Lunch and Learn Sciex Lunch and Learn UCT Lunch and Learn Waters Lunch and Learn ABFT Board Meeting ConEd Committee Meeting WS 8: Method Optimization WS 9: NPS Benzos WS 10: Testimony WS 11: Public Health Labs	Time 7-8:30am 7am-12pm 7am-6pm 8am-12pm 8am-12pm 8am-12pm 8am-12pm 10-10:30am 12-1:30pm 12-1:30pm 12-1:30pm 12-1:30pm 12-1:30pm 12-1:30pm 12-1:30pm 12-1:30pm 12-1:30pm 12-5pm 12:15-1:15pm 1:30-5:30pm 1:30-5:30pm 1:30-5:30pm	Place Atrium Room 06 Concourse Atrium Ballroom B Atrium Ballroom A Room 01 Room 03 Atrium Room 14 Room 14 Room 14 Room 15 Room 15 Room 9 Room 15 Room 9 Room 12 Room 10 Room 11 Room 10 Room 11 Room 16 Room 08 Room 06 Atrium Ballroom B Atrium Ballroom A Room 01 Room 01 Room 04
Iuesday, November 1, 2022 Breakfast (WS Participants Only) SOFT Board Meeting Registration WS 5: New Drugs WS 6: Drugs and Behavior WS 7: Postmortem Alcohols ABFT Exam Break (WS Participants Only) Abbott Lunch and Learn Agilent Lunch and Learn Bruker Lunch and Learn Randox Lunch and Learn Sciex Lunch and Learn Shimadzu Lunch and Learn UCT Lunch and Learn Waters Lunch and Learn ABFT Board Meeting ConEd Committee Meeting WS 8: Method Optimization WS 9: NPS Benzos WS 10: Testimony WS 11: Public Health Labs Break (WS Participants Only)	Time 7-8:30am 7am-12pm 7am-6pm 8am-12pm 8am-12pm 8am-12pm 8am-12pm 10-10:30am 12-1:30pm 12-1:30pm 12-1:30pm 12-1:30pm 12-1:30pm 12-1:30pm 12-1:30pm 12-1:30pm 12-1:30pm 12-5pm 12:15-1:15pm 1:30-5:30pm 1:30-5:30pm 1:30-5:30pm 3:30-4pm	Place Atrium Room 06 Concourse Atrium Ballroom B Atrium Ballroom A Room 01 Room 03 Atrium Room 14 Room 14 Room 13 Room 15 Room 15 Room 9 Room 15 Room 9 Room 12 Room 10 Room 11 Room 10 Room 11 Room 16 Room 06 Atrium Ballroom B Atrium Ballroom A Room 01 Room 01 Room 04 Atrium
Iuesday, November 1, 2022 Breakfast (WS Participants Only) SOFT Board Meeting Registration WS 5: New Drugs WS 6: Drugs and Behavior WS 7: Postmortem Alcohols ABFT Exam Break (WS Participants Only) Abbott Lunch and Learn Agilent Lunch and Learn Bruker Lunch and Learn Randox Lunch and Learn Sciex Lunch and Learn Sciex Lunch and Learn UCT Lunch and Learn Waters Lunch and Learn ABFT Board Meeting ConEd Committee Meeting WS 8: Method Optimization WS 9: NPS Benzos WS 10: Testimony WS 11: Public Health Labs Break (WS Participants Only) Postmortem Committee Meeting	Time 7-8:30am 7am-12pm 7am-6pm 8am-12pm 8am-12pm 8am-12pm 8am-12pm 10-10:30am 12-1:30pm 12-1:30pm 12-1:30pm 12-1:30pm 12-1:30pm 12-1:30pm 12-1:30pm 12-1:30pm 12-1:30pm 12-5pm 12-1:30pm 12-5pm 12-5pm 12:50pm 1:30-5:30pm 1:30-5:30pm 1:30-5:30pm 1:30-5:30pm 1:30-5:30pm 1:30-5:30pm 1:30-5:30pm 1:30-5:30pm 1:30-5:30pm 1:30-5:30pm 1:30-5:30pm 1:30-5:30pm 1:30-5:30pm 1:30-5:30pm 1:30-5:30pm	Place Atrium Room 06 Concourse Atrium Ballroom B Atrium Ballroom A Room 01 Room 03 Atrium Room 14 Room 14 Room 13 Room 15 Room 15 Room 9 Room 15 Room 9 Room 12 Room 10 Room 11 Room 10 Room 11 Room 16 Room 08 Room 08 Room 08 Atrium Ballroom B Atrium Ballroom A Room 01 Room 01 Room 04 Atrium Room 03

6:30-9:30pm

Exhibit Hall A

Welcome Reception

Tuesday, November 1, 2022	Time	Place
	0.00.10	D 01
Elmer Gordon Forum	8:30-10pm	Room U1
MilliporeSigma Nite Owi Reception	TUPM-T2am	Atrium
Wednesday, November 2, 2022	Time	Place
First-Time Attendee Breakfast	7-8am	Room 01
Continental Breakfast	7-8:30am	Atrium
Poster Presentation Prep	7-11am	Exhibit Hall A
Registration	7am-5pm	Concourse
Opening Plenary Speaker	8-9am	Atrium Ballroom
Scientific Session #1	9-10am	Atrium Ballroom
Exhibit Hall	9:30am-4pm	Exhibit Hall A
Refreshment Break	10-10:30am	Exhibit Hall A
Scientific Session #2	10:30am-12pm	Atrium Ballroom
Lunch with Exhibitors	12-2pm	Exhibit Hall A
Poster Session #1	12-2pm	Exhibit Hall A
SOFT 2023 Planning Committee	12:30-1:30pm	Room 06
Scientific Session #3	2-3:30pm	Atrium Ballroom
Refreshment Break	3:30-4pm	Exhibit Hall A
Scientific Session #4	4-5pm	Atrium Ballroom
Oral Fluid Committee	5-6pm	Room 06
Mentoring Program Reception	5-6pm	Room 04
Rock Hall Off-Site Event	7-11pm	Rock & Roll Hall of Fame
Thursday, November 3, 2022	Time	Place
Karla Mooro Eun Pun	6.20 9am	Hilton Jakosida Entranco
Continental Breakfast	7_8·20am	Atrium
AAES Steering Committee	7-9am	Room 04
Poster Presentation Pren	7-11am	Exhibit Hall A
Registration	7 main 7am-1nm	Concourse
Scientific Session #5	8-9:30am	Atrium Ballroom
Exhibitor Feedback Meeting	8:30-9:30am	Room 06
Refreshment Break	9:30-10am	Exhibit Hall A
Exhibit Hall	9:30am-3:30pm	Exhibit Hall A
Scientific Session #6	10am-12pm	Atrium Ballroom
Lunch with Exhibitors	12-2pm	Exhibit Hall A
Poster Session #2	12-2pm	Exhibit Hall A
DFC Committee	12:30-1:30pm	Room 06
CVD Committee	12:30-1:30pm	Room 04
Scientific Session #7	2-3pm	Atrium Ballroom
Refreshment Break	3-3:30pm	Exhibit Hall A
SOFT Business Meeting	3:30-5pm	Atrium Ballroom
Happy Hour	6-7pm	Hilton - 5 th Floor
President's Banquet	7pm-12am	Hilton - 5 th Floor
Friday November 4, 2022	Time	Place
Continental Producest	7 0.20cm	Consource
Scientific Session #2	1-0.3Udill	
3014HIIIIIC 34221011 # 0	9_1(lom	Atrium Rollroom
Refreshment Breek	8-10am	Atrium Ballroom
Refreshment Break	8-10am 10-10:30am 10:30am-12pm	Atrium Ballroom Concourse Atrium Ballroom
Refreshment Break Scientific Session #9	8-10am 10-10:30am 10:30am-12pm 12-12:30pm	Atrium Ballroom Concourse Atrium Ballroom



INGTON NTER CNN LEV E







PRESIDENT ROBERT SEARS



SECRETARY CHRIS HEARTSILL



DIRECTOR MADELEINE J. SWORTWOOD



DIRECTOR MADELINE MONTGOMERY



PRESIDENT ELECT ERIN SPARGO



PAST PRESIDENT AMY MILES



DIRECTOR ANDRE SUKTA



TREASURER TATE YEATMAN



EXECUTIVE DIRECTOR BETH OLSON



DIRECTOR PHILIP M. KEMP



DIRECTOR JERI ROPERO-MILLER



DIRECTOR BILL JOHNSON



COUNSELOR AMANDA J. JENKINS



COUNSELOR DANIEL S. ISENSCHMID

5



2022 CO-HOST SHELLY MERVES CROSBY



2022 CO-HOST DOUG ROHDE

SCIENTIFIC PROGRAM COORDINATORS MOBILE APPLICATION COORDINATORS

MICHELE GLINN KIMBERLY TOMLINSON RUSTY LEWIS ROXANE RITTER SUNDAY HICKERSON

WORKSHOP PROGRAM COORDINATORS

JAYNE THATCHER NATHALIE DESROSIERS

EXHIBITOR LIAISON

LIZ KIELY

FOOD AND BEVERAGE COORDINATORS

ANN MARIE GORDON DELISA DOWNEY DENICE TEEM CARL WOLF

YFT CHAIR Amanda Rausch

AUDIO/VISUAL COORDINATOR

FRANK WALLACE

FUN RUN COORDINATORS ERIC LAVINS KIM YACOUB

JAT SPECIAL ISSUE EDITOR REBECCA HARTMAN

SOFT STAFF

BETH OLSON - EXECUTIVE DIRECTOR CC WATSON - OPERATIONS MANAGER



Thank you to our wonderful reviewers for volunteering their time to review this year's abstract submissions. We appreciate you and your dedication to our toxicology community.

Dustin Abbott Teeshavi Acosta **Robert Almeida** William Anderson Luigino Apollonio **Rachel Barnett** Katherine Bollinger James Burris Sarah Buxton de Quintana Amanda Cadau Jared Castellani Michael Chen Kacey Cliburn Marta Concheiro-Guisan Christopher Cording Joseph Cox Donna Coy Susan Crumpton Nathalie Desrosiers **Katherine Dozier** William Dunn Kayla Ellefsen Michael Fagiola **Kenneth Ferslew** Marissa Finkelstein Steven Fleming Melissa Fogarty Dwain Fuller Michele Glinn Oliver Grundmann Jeffery Hackett Sarrah Hannon Veronica Hargrove Rebecca Hartman **Brendan Heffron Kimberley Heine** Theresa Hippolyte-Olsen Jennifer Hobbs Kayla Horst

Amanda Jenkins **Bill Johnson Robert Johnson** Kristin Kahl Erin Karschner Graham Kennedy **Carrie Kirkton** Samuel Kleinman Peter Koin Ann-Sophie Korb Alex Krotulski John Kucmanic Gary Kunsman **Eric Lavins** Dayong Lee Stephanie Marco Laureen Marinetti Afton Martinez María A. Martinez Peter Maskell Dani Mata **Gregory McIntire** Vanessa Meneses Garry Milman Tonya Mitchell Celia Modell Amanda Mohr Madeline Montgomery Colleen Moore **Tiffany Moreno** Carrol Nanco Kayla Neuman Stephanie Olofson **Kaitlyn Palmquist Rebecca Phipps** Erika Phung Justin Poklis Harry Qui Sumandeep Rana

Luke Rodda **Corissa Rodgers Tim Rohrig Bryan Ruggery** Joseph Saady Alberto Salomone **Kim Samano** Alexander San Nicolas Sara Schreiber William Schroeder Karen Scott **Robert Sears Kevin Shanks** Aaron Shapiro Elisa Shoff Irene Shu **Dennis Siewert Elizabeth Smalley** Erin Spargo Jon Stephenson Kristen Steward Erin Strickland Michael Stypa Madeleine Swortwood **Casey Talastas** Mike Tanner **Denice** Teem Nicholas Tiscione **Kimberly Tomlinson** Michael Truver Manoj Tyagi Kelly Virkler Frank Wallace Darcie Wallace Duckworth Carl Wolf Lauren Wolfe Tate Yeatman **Bob Zettl**

WORKSHOP CHAIRS

Justin Brower Kayla Ellefsen Joe Kahl Robert Kronstrand Alex Krotulski Dani Mata Vanessa Meneses Amy Miles Kayla Neuman Sue Pearring Jason Peterson Alberto Salomone Karen Scott Kevin Shanks Aaron Shapiro Christophe Stove Nicholas Tiscione Rebecca Wagner Karen Woodall

MODERATORS

Kayla Ellefsen Michele Glinn Marilyn Huestis Bill Johnson Matthew Juhascik Joe Kahl Erin Karschner Melissa Kennedy Liz Kiely Marc LeBeau Jen Limoges Dani Mata Amy Miles Madeline Montgomery Steven Raso Lisa Reidy

JAT REVIEWERS

Tim Rohrig Elisa Shoff Madeleine Swortwood Kimberly Tomlinson Courtney Wardwell

Roxane Ritter

William Anderson Dan Anderson Sarah Bartock Sandra Bishop-Freeman Diane Boland Marisol Castaneto Jennifer Colby Marta Concheiro Gail Cooper Michael Corbett Janine De King

Sara Dempsey Brigitte Desharnais Nathalie Desrosiers Kayla Ellefsen Kenneth Ferslew Melissa Fogarty Dwain Fuller Teresa Gray Chris Heartsill Bradford Hepler Daniel Isenschmid Amanda Jenkins Robert Johnson William Johnson Erin Karschner Pascal Kintz Robert Kronstrand Alex Krotulski Gary Kunsman Dayong Lee Wayne Lewallen Russell Lewis Dani Mata Kari Midthun Christine Moore Haley Mulder Lorna Nisbet Justin Poklis Suman Rana Stephen Raso Lisa Reidy Luke Rodda Timothy Rohrig Eugene Schwilke Irene Shu Madeleine Swortwood Nicholas Tiscione Michael Truver Svante Vikingsson Tate Yeatman







RANDOX TOXICOLOGY



The Power of Precision







SOFT EVENTS SPONSORED BY TIER I SPONSORS INCLUDE:

WELCOME RECEPTION WITH THE EXHIBITORS ELMER GORDON FORUM PRESIDENT'S COCKTAIL HOUR & BANQUET RECEPTION MEETING LUNCHEONS REFRESHMENT BREAKS CONTINENTAL BREAKFASTS













UTAK Waters

SOFT EVENTS SPONSORED BY TIER II SPONSORS INCLUDE:

WELCOME RECEPTION WITH THE EXHIBITORS ELMER GORDON FORUM PRESIDENT'S COCKTAIL HOUR & BANQUET RECEPTION MEETING LUNCHEONS REFRESHMENT BREAKS CONTINENTAL BREAKFASTS



clinisys





SOFT EVENTS SPONSORED BY TIER III SPONSORS INCLUDE: CONTINENTAL BREAKFASTS & REFRESHMENT BREAKS MEETING LUNCHEONS



ARK DIAGNOSTICS, INC. BIOTAGE, LLC INDIGO BIOAUTOMATION TECAN

SOFT EVENTS SPONSORED BY TIER IV SPONSORS INCLUDE:

REFRESHMENT BREAKS CONTINENTAL BREAKFASTS

JEOL USA, INC NEOGEN PEAK SCIENTIFIC INC

SOFT EVENTS SPONSORED BY TIER V SPONSORS INCLUDE: CONTINENTAL BREAKFASTS





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

Symposium and Professional Development Fair (PDF) Sunday, October 30, 4:30-9:00 PM

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

Agenda

4:30-5:30 pm	Professional Development Fair/Social Networking
5:30-5:40 pm	YFT Committee Introductions
5:40-6:25 pm	Symposium/PDF Speaker: Dr. Teri Stockham - The Business of Being An Expert Witness
6:25-6:35 pm	SOFT Board Introductions
6:35-7:00 pm	Icebreaker
7:00-7:15 pm	Break
7:15-7:45 pm	2021 Leo Dal Cortivo Award Winners: Sara E. Walton and Ludmyla S. Tavares
7:45-9:00 pm	Open Forum Discussion

Student Enrichment Program (SEP) Monday, October 31, 8:00 AM – 5:00 PM

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

Leo Dal Cortivo Award Thursday, November 3, 7:00 -8:30 PM

The Leo Dal Cortivo Memorial Fund allows the YFT committee to present two awards, each with a cash prize of \$1,000 in addition to free registration at a future SOFT meeting. One award will be presented to the best poster presentation and the other for the best oral presentation. The 2022 winners will be announced at the President's Banquet on Thursday, November 3.





YOUNG FORENSIC TOXICOLOGISTS SPEAKER SUNDAY, OCTOBER 30 4:30-9:00 PM



TERI STOCKHAM, M.S.F.S., PH.D.

Dr. Teri Stockham is a nationally respected consultant, lecturer and author on the topic of forensic toxicology that includes postmortem, human performance and workplace toxicology. Over the past 35 years, she has gained extensive education, training and experience both in the laboratory and the courtroom. She is currently president and owner of her own consulting firm, Teri Stockham, Ph.D., Inc.

Dr. Stockham is one of few experts in the nation who has earned three degrees focused on the specialty of forensic toxicology. She achieved her training, experience and expertise working for a number of medical examiner offices, including six years as Chief Toxicologist in Broward County, Florida. She served three years with the Office of Chief Medical Examiner in New York City as well as time with the medical examiners office in Richmond, Virginia. Additionally, she has been a consultant for Kroll/PharmChem Laboratories providing court testimony regarding urine test results for violation of probation and parole.

Dr. Stockham earned her doctoral degree from the Medical College of Virginia in Pharmacology and Toxicology, and her M.S. degree in Forensic Science from The George Washington University. Her undergraduate studies in chemistry were completed at Indiana State University, where she graduated Magna Cum Laude.

Since 1997, Dr. Stockham's consultation has been sought in a wide variety of court cases including personal injury, driving under the influence (DUI), insurance defense, worker's compensation, probation/parole, child custody, drug-facilitated sexual assault and court-martials. Her career as a consultant has included a wide variety of clients, both plaintiff and defense law firms who represent individuals, insurance companies, health-care providers, business entities and various government and military agencies.

Her professional memberships include the Society of Forensic Toxicologists (SOFT), American Academy of Forensic Sciences (AAFS) and The International Association of Forensic Toxicologists (TIAFT).





DR. KARLA MOORE

Join fellow SOFT attendees on Thursday, November 3 from 6:30-8 AM for the Annual Karla Moore Memorial Tox 'N Purge Fun Run/Walk!

The original Tox 'N Purge run was created by Dr. Karla Moore in 1997 for the Salt Lake City meeting. In addition to her involvement in the field of toxicology and participation in SOFT, she was an officer in the United States Air Force. After her passing in 2008, the run was memorialized in her honor.

The proceeds from the run are donated to the American Cancer Society in Dr. Moore's memory. Expenses for the event are supported by our SOFT exhibitors.

2022 FUN RUN SPONSORS















Thank you to our 2022 ERA Donors! The purpose of the award is to encourage academic training and research in areas related to forensic toxicology and recognize worthy student researchers. The award is to be used to assist the awardee with travel expenses to attend the Annual SOFT Meeting for the purpose of presenting their research.

Ahmed Al-Asmari William H. Anderson Dan T. Anderson Timothy A. Appel Sarah Bartock Michael Baylor Sandra Bishop-Freeman Donna Bush Marisol Castaneto Ayako Chan-Hosokawa Jennifer Colby Edward J. Cone Anthony G. Costantino Michael J. Coyer Susan Crumpton Nathalie Desrosiers Julia Diaz Laurel Farrell Dwain C. Fuller Diana Garside Demetra (Demi) Garvin Dimitri Gerostamoulos Ann Marie Gordon Teresa Gray Dale Hart Rebecca L. Hartman Huda Hassan Chris Heartsill Bradford R. Hepler Marilyn Huestis William R. Johnson Robert D. Johnson Sabra R. Jones Prentiss Jones, Jr. Erin Karschner Sean E. Kocur Ann-Sophie Korb James Kraner **Robert Kronstrand** Marc A. LeBeau Dayong Lee

Nikolas P. Lemos Corey Lightfoot Ray Liu Danielle Mackowsky Maria A. Martinez Sam Mathews Michael Mbughuni **Diane Mertens-Maxham** Amy Miles Adam Negrusz Maria Oliveras Alex Pappas **Michelle Peace** Patricia Pizzo Luke N. Rodda Jeri D. Ropero-Miller Joseph J. Saady Alberto Salomone Tania Sasaki **Eugene Schwilke** Karen Scott Robert M. Sears Danielle E. Serena Clementz Matt Slawson Michael L. Smith Chetan Soni Erin A. Spargo **Elizabeth Spratt Robert Sroka** Peter R. Stout Erin C. Strickland Andre Sukta **Craig Sutheimer** Madeleine J. Swortwood Jayne Thatcher Samantha Tolliver Javier Velasco **Bridget Verdino** Svante Vikingsson Jeff Walterscheid **Diana Wilkins**



OPENING PLENARY SPEAKER WEDNESDAY, NOVEMBER 2 8:00-9:00 AM



JOLENE DEFIORE-HYRMER, MPH

Jolene DeFiore-Hyrmer, MPH is the Bureau Chief of the Bureau of Health Improvement and Wellness at the Ohio Department of Health. As the chief administrator she oversees multiple programs including Chronic Disease Prevention, Chronic Disease Epidemiology and Evaluation, Cancer Prevention and Surveillance, Health Promotion, Tobacco Use Prevention and Cessation, Office of Primary Care, State of Office of Rural Health, and Violence and Injury Prevention and Surveillance. Ms. DeFiore-Hyrmer is the current principal investigator of Ohio's CDC Overdose Data to Action Cooperative Agreement, and has served as principal investigator of multiple grants including Ohio Violent Death Reporting System, the Prevention of Prescription Drug Overdose Program, Enhanced Surveillance of Ohio Opioid-Involved Morbidity and Mortality and the State Core Violence and Injury Prevention Program grants funded through the Centers for Disease Control and Prevention.

Ms. DeFiore-Hyrmer has overseen the implementation of multiple public health surveillance systems and authored multiple surveillance documents. She is also responsible for the implementation of injury prevention projects, including expansion of community-based interventions, Project DAWN (Deaths Avoided With Naloxone), and other initiatives focused on the prevention of injuries. Ms. DeFiore-Hyrmer has served on numerous state committees and advisory groups such as the Ohio Overdose Prevention Network, Second Chance Trust Advisory Committee, Ohio Trauma Committee, and the RecoveryOhio Interagency Technical Working Group.



Workshop 2: Work Smarter, Not Harder – Process Improvements and Automation Date: Monday, October 31, 2022 Time: 8:00 AM-5:30 PM Chairs: Aaron Shapiro and Sue Pearring

Today's high throughput laboratories utilize advanced automation systems for laboratory processes including sample handling, instrument analysis, and data processing. While many forensic laboratories lack either the resources (whether financial or skill-based) or the throughput to justify the costs of implementing such systems, there are a number of processes that can be improved in a forensic laboratory with minimal or no capital investment using simple scripts in Microsoft Excel or RStudio.

This workshop will showcase examples of automation on large and small scales and discuss tools for developing a successful business case to garner support from your administrators. Additionally, this workshop will feature a unique master class where participants will have the opportunity to improve existing processes with the collaboration of presenters and fellow attendees.

Workshop 3: There's Something About Fentanyl Date: Monday, October 31, 2022 Time: 8:00 AM-5:30 PM Chairs: Kevin Shanks and Justin Brower

Novel psychoactive substances (NPS) dominate the conversation, but the elephant in the room is fentanyl. The current opioid epidemic was initially tied to prescription opioids, but over the last several years, drug overdose rates have skyrocketed due to illicitly manufactured (non-pharmaceutical) opioids. Today, fentanyl is one of the most commonly detected substances in forensic toxicology laboratories. In this full-day comprehensive workshop, There's Something About Fentanyl, we will discuss the current state of knowledge surrounding fentanyl – its beginnings and illicit synthesis, trends, screening strategies in the toxicology laboratory, postmortem interpretation, human impairment, and the fentanyl crisis from both a clinician's and a medical examiner's perspective.

Workshop 4: Method of Standard Addition for Analyte Quantification for Application to Postmortem Matrices and Novel Psychoactive Substances

Date: Monday, October 31, 2022 Time: 1:30 PM-5:30 PM Chairs: Joe Kahl and Alex Krotulski

The SOFT Postmortem Committee and Novel Psychoactive Substances (NPS) Committee presents a collaborative workshop revolving around implementation and application of method of standard addition (MSA). MSA is a well-defined and well-studied approach for quantifying analytes in a variety of matrices. While MSA is somewhat of an emerging aspect in forensic toxicology, its history dates back several years. While there can be many approaches to the manner in which MSA is applied, the most common approach used in forensic toxicology is an internal calibration model, which differs from an external calibration model (calibration curve and controls) that is used most commonly in most forensic laboratories. In simple terms, MSA uses the sample matrix itself as the matrix fortified for quantitative assessment, and the general process involves the addition of increasing concentrations of analyte reference material to the sample matrix in a set number of sample replicates. Due to known issues with matrix effects, MSA has been routinely employed for the analysis of analytes in postmortem tissues for many years. With the ease of method development and verification, MSA has more recently been applied to the quantitation of NPS in a variety of case types which has allowed some toxicology laboratories to better keep pace with the constant evolution of the synthetic drug market and its short life cycles. These advantages, as well as some disadvantages, will be discussed during this workshop.

Workshop 5: New Drugs Require New Approaches in Forensic Toxicology Date: Tuesday, November 1, 2022 Time: 8:00 AM – 12:00 PM Chairs: Christophe Stove and Alberto Salomone

Forensic toxicology aims to apply newly developed methods in the field or inside the laboratory, in order to resolve complex intoxications or judicial cases. Object of the analysis can be either a biological sample or a seized material. Quite often, only small quantities of sample are available, in which the analyte is present at trace level. Furthermore, new and unknown compounds are continuously introduced onto the black market, introducing a risk of 'missed cases'. In this scenario, innovation is highly needed in forensic toxicology, in terms of more sophisticated methods,

improved access to alternative matrices, advancement of multivariate statistical and machine learning tools. Only a proper analytical approach and a combination of analysis and processing of results can, indeed, assist the interpretation of the findings and support the case solving in real situations.

Workshop 6: Drugs and Behavior: The Role of Forensic Toxicology Date: Tuesday, November 1, 2022

Time: 8:00 AM – 12:00 PM

Chairs: Karen Woodall and Aaron Shapiro

Psychopharmacology is defined as the study of drug-induced changes in mood, thinking and behavior. Forensic toxicologists are frequently required to provide opinions about psychoactive drugs when the behavioral effect on an individual is an important issue. This workshop aims to provide an overview on the topic of psychopharmacology and the types of cases that often require an opinion from a forensic toxicologist. Cases may include homicide investigations, drug facilitated sexual assaults, or death investigations where it is the behavior of the deceased that is relevant to determining the manner of death. In addition, the types of questions that may be asked during testimony during a criminal trial and how to stay within the scope of your expertise will be discussed.

Workshop 7: Analysis and Interpretation of Postmortem Alcohols Date: Tuesday, November 1, 2022 Time: 8:00 AM-12:00 PM Chairs: Robert Kronstrand and Karen Scott

Ethanol is one of the most commonly detected drugs in postmortem cases, which makes it an important substance to analyze for and to interpret it correctly. The "gold standard" for quantitative determination of ethanol is gas chromatography with a flame ionization detector using columns with different selectivity, but the alcohols can also be identified and quantified using mass spectrometry. Methods commonly include ketones and higher alcohols to monitor markers for acidosis or postmortem formation of ethanol. Analysis of acetone serves as a means to screen for possible acidosis and can prompt for additional analysis to differentiate between alcohol-induced ketoacidosis, diabetic ketoacidosis as well as hyper osmolar syndrome. In addition to the oxidative metabolism of ethanol to acetaldehyde and acetic acid, there are also minor non-oxidative pathways including conjugation with glucuronic acid and sulphate. Even though minor, these pathways can be helpful in interpreting postmortem formation of ethanol. At present, the analysis of two or more matrices is commonly used to investigate postmortem formation but these minor metabolites, and the analysis of the higher alcohols, 1-propanol and 1-butanol may better help the toxicologist in interpreting postmortem ethanol findings. Chronic excessive alcohol consumption is an underlying cause of death, but diagnosis is difficult. However, objective measures are available and include the analysis of hair for ethyl glucuronide and blood for phosphatidyl ethanol. In this workshop we will address the biochemical and analytical aspects of alcohols in postmortem case work. We will evaluate different approaches to investigate postmortem formation of ethanol and chronic alcohol consumption.

Workshop 8: Method Optimization During Development and Validation: Tips and Tricks on How to Succeed

Date: Tuesday, November 1, 2022

Time: 1:30 AM-5:30 PM

Chairs: Rebecca Wagner and Dani Mata

The SOFT Applied Analytical Toxicology Committee presents its first workshop offering strategies associated with method development and validation. The publication of ANSI/ASB Standard 036 Standard Practices for Method Validation in Forensic Toxicology has resulted in laboratories revisiting their methodologies to be in accordance with the standard. However, many laboratories are not equipped with the staff to perform in-depth method development or validations. This half-day workshop will cover aspects of method development and validation to assist scientists in performing validations efficiently and with limited setbacks. The workshop will cover a brief introduction into ANSI/ASB Standard 036 Standard Practices for Method Validation in Forensic Toxicology and focus on the technical aspects of method development and validation. Topics will include appropriate selection of sample preparation and instrumentation, matrix considerations, automated sample preparation considerations, and the verification process of transferring methods to additional instrumentation. Additionally, the workshop will include practical exercises to allow engagement between the speakers and workshop participants. Method development and validation can be a challenging task for many laboratories. Upon completion of the workshop, participants will have a stronger foundation in understanding the common problems that may arise during development/validation and be provided with guidance and strategies to minimize these difficulties.

Workshop 9: Driving Under the Influence: NPS Benzodiazepines Date: Tuesday, November 1, 2022 Time: 1:30-5:30 PM Chairs: Kayla Ellefsen and Nicholas Tiscione

Despite active government regulation of novel psychoactive substances (NPS), new compounds with unknown pharmacology and toxicities are continuously emerging, resulting in adverse events and increased prevalence in driving under the influence of drugs (DUID) investigations. These purported "legal highs" pose problems for law enforcement, military, and public health and safety officials, along with toxicologists who must identify an unending variety of new drugs of abuse. NPS benzodiazepines are a class of NPS that continues to grow and increase in prevalence. Oftentimes, drug users may be unaware they are taking NPS Benzodiazepines as they are frequently found in counterfeit tablets marketed as traditional benzodiazepines. Although an increased number of these compounds are being detected as our knowledge and analytical methods expand, interpretation of NPS benzodiazepines may pose challenges to forensic toxicologists appearing in courts to discuss their findings in relation to DUID cases. The SOFT NPS Committee and the SOFT/AAFS Drugs and Driving Committee brings to you a joint workshop on driving under the influence of NPS benzodiazepines. The workshop will discuss current trends of NPS benzodiazepines, analytical methods to detect and quantify these compounds in DUID cases, and concentrations observed in DUID populations. Additionally, the workshop will cover their pharmacology and general physiological and behavioral effects, presented through case reports. Finally, strategies for trials involving NPS benzodiazepines and challenges associated with interpreting and testifying on NPS benzodiazepine DUID cases will be explored.

Workshop 10: Good Reputation: The Beginner's Guide to Toxicology Testimony Date: Tuesday, November 1, 2022 Time: 1:30-5:30 PM

Chairs: Kayla Neuman and Vanessa Meneses

In this half-day workshop, participants will be given a look into all aspects of providing testimony as an expert in both human performance and postmortem toxicology. Presentations by attorneys and toxicologists will cover topics including courtroom basics, curriculum vitae preparation, pre-trial preparation, overview of current guidelines for testimony in forensic toxicology, and interpretation of toxicological findings for a jury. This workshop will conclude a Q&A session with all presenters.

Workshop 11: Spotlight on Public Health Labs – Opportunities for Knowledge Transfer Between the Forensic and Public Health Community

Date: Tuesday, November 1, 2022

Time: 1:30-5:30 PM

Chairs: Amy Miles and Jason Peterson

In this workshop, attendees will learn about the testing and surveillance activities undertaken by public health laboratories related to surveillance of opioid overdoses and other emerging chemical threats, as well as gain a valuable medical toxicology perspective on laboratory results interpretation. Attendees will be given an opportunity to explore areas in which forensic laboratory and public health laboratory goals and processes overlap and network/learn from one another, identifying opportunities for knowledge transfer. We are happy you are here with us in Cleveland! Below is an overview of SOFT's social events. You can always stop by the registration desk if you have any questions.

YFT Symposium Sunday, October 30, 4:30-9:00 pm Atrium Ballroom

The YFT Symposium is hosted by our Young Forensic Toxicologists Committee. The committee is responsible for planning the evenings events and selecting our guest speaker. Please join them for the Professional Development Fair, an Icebreaker activity, and presentations from the 2021 Dal Cortivo winners. You must be 41 years or younger to attend the symposium. Food and beverage are provided to attendees.

Lunch and Learns Monday, October 31 and Tuesday, November 1

Join our 2022 sponsors at a lunch and learn! This is a wonderful opportunity to have lunch and learn about a topic related to toxicology. Please see the schedule for sponsor lunch and learn room information.

Thermo Fisher Scientific."Rock & Skull Karaoke Reception" Monday, October 31st, 7:30-10:00 PM Atrium

Get your "Rock & Skull" on this Halloween with Thermo Fisher Scientific! We want to see you in your best costume as you perform your favorite rock and roll songs! We'll provide the food and drinks, and you bring your star power. Commemorate the night with our Halloween-inspired photobooth & prize giveaways! You will not want to miss this fun and exciting event!

Welcome Reception Tuesday, November 1, 6:30-9:30 pm Exhibit Hall A

Please join us in the Exhibit Hall for a warm welcome with the exhibitors. Tour the hall and stop in at your favorite exhibitor's booth. Food and beverage are included for the following registration types: Member, Student, Non-member, Exhibitor, Accompanying Person, and Daily. You can find that hall map on page 228.

MilliporeSigma Nite Owl Reception Tuesday, November 1, 10:00-12:00 pm Atrium

Join us at the Nite Owl Reception hosted by Tier II sponsor, MilliporeSigma! Halloween is not over!!! Dress up as your favorite musician and Let's rock 'n roll with MilliporeSigma all night long.

Rock and Roll Hall of Fame Off-Site Event Wednesday, November 2, 7:00-11:00 pm Rock and Roll Hall of Fame

Join us for a night out at the Rock and Roll Hall of Fame. Please meet at the Hilton Lakeside Entrance for transportation to the Rock Hall. The Rock Hall is a 1/2 mile from the convention center. For those who prefer to travel by bus, please meet at the Lakeside Entrance of the Hilton. See map on page 3. Food and beverage are provided at the venue for the following registration types: Member, Student, Non-member, Exhibitor and Accompanying Person.

President Sears' Red Carpet Banquet Thursday, November 3, 6:00 pm - 12:00 am Hilton - 5th Floor

Enjoy a plated dinner, drinks, and dancing! Attendees are encouraged to wear cockatil attire and have their picture taken on the red carpet. The banquet includes the following registration types: Member, Student, Non-member, Exhibitor and Accompanying Person.

PLATFORM PRESENTATION SCHEDULE

SCIENTIFIC SESSION 1

Wednesday, November 2

9:00-10:00 am

Atrium Ballroom

Moderators: Lisa Reidy and Marc LeBeau

Topics: Alternative Matrices and Drug Facilitated Crimes

Time	Platform Number	Title	Speaker
8:00-9:00 am		Plenary Speaker - Jolene DeFiore-Hyrmer, MF	РН
9:00-9:15 am	S1	Statistical Comparison of Optimized and Least Ef- fective Forensic Hair Analysis Methods for Multiple Drugs and Metabolites in Authentic User Specimens	Brianna Spear
9:15-9:30 am	S2	Improved Automated Extraction and High-Through- put Analysis of Δ9-Tetrahydrocannabinol (Δ9-THC) in Oral Fluid	Omran Muslin
9:30-9:45 am	S3	Examination of Acetone Findings in Suspected Drug-Facilitated Sexual Assault	Crystal Arndt
9:45-10:00 am	S4	DFC Casework vs Public Perception of DFC Drugs – Fact and Fiction?	Meaghan Ringel
10:00-10:30 am - Morning Break			

SCIENTIFIC SESSION 2

Wednesday, November 2

10:30 am - 12:00 pm

Atrium Ballroom

Moderators: Joe Kahl and Jen Limoges

Topic: Postmortem

Time	Platform Number	Title	Speaker		
10:30-10:45 am	S5	Prevalence of Xylazine in Overdose Cases: An Anal- ysis of Miami-Dade County Medical Examiner Case Data	Rocio Potoukian		
10:45-11:00 am	S6	Fatal Overdose Involving the Organophosphate In- secticide Malathion	Stephanie M. Marco		
11:00-11:15 am	S7	The Relationship between Acetone and β-hydroxy- butyrate (BHB) Levels in Postmortem Toxicology Casework	William Schroeder II		
11:15-11:30 am	S8	Mitragynine-only deaths in North Carolina	Justin Brower		
11:30-11:45 am	S9	Fatal Intoxication with O-Desmethyltramadol (ODS- MT)	Laureen J. Marinetti		
11:45 am-12:00 pm	S10	A 5-Year Review of PCP-Related Deaths in Dallas County	Lindsay Glicksberg		
	12:00-2:00 pm - Lunch				

Wednesday, November 2

2:00-3:30 pm

Atrium Ballroom

Moderators: Erin Karschner and Bill Johnson

Topic: Drugs and Driving Special Session

Time	Platform Number	Title	Speaker
2:00-2:15 pm	S11	Evaluating Drug Positivity for Tier I and Tier II Drugs Relative to BAC Thresholds	Amanda Mohr
2:15-2:30 pm	S12	Regional Toxicology Liaison Demonstration Project	Kristen Burke Chris Heartsill Sabra Jones
2:30-2:45 pm	S13	Rapid Extraction and Qualitative Screening of 30 Drugs in Oral Fluid at Concentrations Recommended for the Investigation of DUID Cases	Cynthia Coulter
2:45-3:00 pm	S14	Validation of Novel Cannabinoids including Δ 8-THC, Δ 10-THC, THC-O and THC-P in Blood and Oral Fluid for DUID Testing	Kristin Tidwell
3:00-3:15 pm	S15	Double Back - Two DUI cases involving Lormetaze- pam, Lorazepam, and Fentanyl	Stephanie Olofson
3:15-3:30 pm	S16	Evaluating the Practice of Triple Draws in Colorado with the OSAC Guidelines for Performing Alcohol Calculations in Forensic Toxicology	Vanessa Beall
		3:30-4:00 pm - Afternoon Break	

SCIENTIFIC SESSION 4

Wednesday, November 2

4:00-5:00 pm

Atrium Ballroom

Moderators: Amy Miles and Melissa Kennedy

Topic: Human Performance

Time	Platform Number	Title	Speaker
4:00-4:15 pm	S17	Vaping ethanol: roadside investigations, clinical im- plications, and subjective effects	Alaina Holt
4:15-4:30 pm	S18	Xanax and Driving: An Attempted Suicide Defense Case Study.	Curt E. Harper
4:30-4:45 pm	S19	The Effects of COVID-19 on Fentanyl Concentrations in DUID and PM Cases in Orange County, CA from 2018 - 2021	Dani Mata
4:45-5:00 pm	S20	Impaired Driving Trends Before and During the COVID-19 Pandemic in Houston, Texas	Jami Reber

Thursday, November 3

8:00-10:00 am

Atrium Ballroom

Moderators: Dani Mata and Kayla Ellefsen

Topics: Novel Psychoactive Substances and Clinical Toxicology

Time	Platform Number	Title	Speaker
8:00-8:15 am	S21	First Identification, Chemical Analysis and Pharmaco- logical Characterization of N-piperidinyl Etonitazene (etonitazepipne), a Recent Addition to the 2-benzyl- benzimidazole Opioid Subclass	Marthe Vandeputte
8:15-8:30 am	S22	Evaluating Cross Reactivity of New Psychoactive Sub- stances (NPS) on Immunoassay in Whole Blood	Grace Cieri
8:30-8:45 am	S23	Red Herrings, White Lies, and Blue Lotus	Joshua Seither
8:45-9:00 am	S24	Designer Benzodiazepine Trends in Northeast Ohio: November 2019 - March 2022	Kimberly Yacoub
9:00-9:15 am	S25	Quantitation of the New Synthetic Cathinone N,N-Dimethylpentylone in a Post-Mortem Case Series	Melissa F. Fogarty
9:15-9:30 am	S26	"Diet-Weed's" (Δ8-THC) cross-reactivity with Six Commercial Cannabinoid Urine Screening Kits	Ashley A. Pokhai
9:30-9:45 am	S27	Benzo-Dope: An Increasingly Prevalent Drug Combi- nation of Significant Toxicological Relevance	Alex J. Krotulski
9:45-10:00 am	S28	Hydromorphone and Hydromorphone-3-glucuro- nide in Blood: Environmental Impact of Developed Procedure	Christine Moore
		10:00-10:30 am - Morning Break	

SCIENTIFIC SESSION 6

Thursday, November 3

10:30 am - 12:00 pm

Atrium Ballroom

Moderators: Matthew Juhascik and Marilyn Huestis

Topic: Analytical Toxicology

Time	Platform Number	Title	Speaker
10:30-10:45 am	S29	Challenges Associated with Cross-Validating Analyt- ical Methods across Multi-Generational LC/MS/MS Platforms in the Forensic Toxicology Laboratory	Britni Skillman
10:45-11:00 am	S30	Getting High (Throughput) on Psilocin	Glenna Brown
11:00-11:15 am	S31	Development of a Supported Liquid Extraction High Resolution Mass Spectrometry-based Drug Screen- ing Protocol for Comprehensive Toxicological Analy- sis of Whole Blood	Jessica Lynn Ayala

11:15-11:30 am	S32	Using the EZSTATSG3 validation tool to make your life easier and your validations robust	Jocelyn Abonamah
11:30-11:45 am	S33	Use of Common Fragments and Neutral Losses to Streamline Screening Workflows for the Detection of Novel Psychoactive Substances in Forensic Toxicolo- gy Casework	Joshua S. DeBord
11:45 am-12:00 pm	S34	Analysis of Commercial Δ8-THC Products for Micro- biologics and Phytocannabinoid Concentrations	Kimberly Karin
		12:00-2:00 pm - Lunch	

Thursday, November 3

2:00-3:00 pm

Atrium Ballroom

Moderators: Tim Rohrig and Elisa Shoff

Topics: ERA and YSMA Award Recipients

Time	Platform Number	Title	Speaker	
2:00-2:15 pm	S35	The Aerosolization Efficiency of Cocaine in a Eutectic Mixture with Nicotine in Electronic Cigarettes	Laerissa Reveil	
2:15-2:30 pm	S36	Pharmacology and Toxicology of N-pyrrolidino Etoni- tazene – a New Nitazene Synthetic Opioid Increas- ingly Observed in Forensic Cases	Sara Walton	
2:30-2:45 pm	S37	Developing a Unified Method for the Analysis of Five Ethanol Metabolites: EtG, EtS, GTOL, 5-HTOL, and 5-HIAA using UPLC-MS/MS	Bailey Jones	
2:45-3:00 pm	S38	Opioid-like Adverse Effects of Tianeptine in Male Rats and Mice	Tyson Baird	
3:00-3:30 pm - Afternoon Break				

SCIENTIFIC SESSION 8

Friday, November 4

8:00-10:00 am

Atrium Ballroom

Moderators: Madeleine Swortwood and Roxane Ritter

Topics: Postmortem and Analytical Toxicology

Time	Platform	Title	Speaker
	Number		
8:00-8:15 am	S39	Identification of 11-nor-D-8-tetrahydrocannabi- nol-9-carboxylic acid in Postmortem Urine	Michele Crosby
8:15-8:30 am	S40	Ohio Poisoning Homicides: A Toxic Bittersweet His- tory	Carrie Diane Mazzola
8:30-8:45 am	S41	Fentanyl Postmortem Redistribution: A Large Popu- lation Review	Erin Strickland

8:45-9:00 am	S42	Babies and Drugs: Drugs Detected in Miami-Dade County Infant Deaths	Jennifer Gonyea
9:00-9:15 am	S43	Missed Opportunities in Medicolegal Drug Death Investigations	Sara Schreiber
9:15-9:30 am	S44	Analytical Challenges Associated with the Quanti- tative Analysis of Tetrahydrocannabinol Isomers in Biological Matrices	Rebecca Wagner
9:30-9:45 am	S45	Changing Landscape of Marijuana Products as Evi- dent from Biological Sample Testing	Sumandeep Rana
9:45-10:00 am	S46	Development and Optimization of a Large Urine An- tipsychotic Drug Panel and Observations of Clozap- ine Internal Standard Enhancement	Theresa Meli
10:00-10:30 am - Morning Break			

Friday, November 4

10:30 am - 12:00 pm

Atrium Ballroom

Moderators: Michele Glinn and Kimberly Tomlinson

Topics: Novel Psychoactive Substances and Other Topics

Time	Platform Number	Title	Speaker
10:30-10:45 am	S47	The SOFT Professional Mentoring Program: Forward Momentum	Helen Ha
10:45-11:00 am	S48	Standards Development Activities in Forensic Toxi- cology	Marc A. LeBeau
11:00-11:15 am	S49	Synthetic Cannabinoids in Human Performance and Postmortem Casework from 2019-2022	Erin L. Karschner
11:15-11:30 am	S50	A Snapshot in Time—2018-2021: Rise and Fall of Substances by Monitoring NFLIS-Drug	DeMia Pressley
11:30-11:45 am	S51	Nitazenes Are Coming To Town: Development of a Qualitative Screening Method for Benzimidazole Opioids and Other Synthetic Opioid Compounds in Postmortem Specimens using LC-QQQ-MS/MS	Elisa N. Shoff
11:45 am-12:00 pm	S52	Fluorofentanyl Detection by LC-QToFMS and Preva- lence in Postmortem Toxicology	Kevin G. Shanks

POSTER PRESENTATION SCHEDULE

POSTER SESSION #1

Wednesday, November 2, 12:00-2:00 pm

Moderators: Madeline Montgomery and Courtney Wardwell

Exhibit Hall A

Poster Number	Title	Speaker
P1	Development of a solid phase extraction method for fentanyl analogs in biological matrices for analysis by LC-MS/MS	Alli Timmons
P2	ELISA detection of 7 PCP analogs in whole blood using Immunalysis phencyclidine (PCP) Direct ELISA Kit	Alyssa Austin
P3	Expanding NPS screening capabilities in the forensic toxicology labora- tory	Casey Burrows
P4	Two-year prevalence of NPS in Québec (Canada): the success of a dy- namic analytical method paired to an internal early warning system	Souryvanh Nirasay
P5	Pharmacological characterization of new, generic ban evading synthetic cannabinoids	Christophe Stove
P6	Quantitative analysis of novel psychoactive substances in various post- mortem matrices using standard addition	Claire Kaspar
Ρ7	Investigative study of synthetic benzodiazepine detection using the Im- munalysis© benzodiazepine ELISA kit in correlation with triple quadru- pole LC/MS/MS and QTOF-LC/MS	Connie Alexia Lewis
P8	Leveraging sensitivity improvements for low-level detection of drugs, metabolites, and endogenous hormones in complex biological matrices	Crystal Holt
P9	The method of standard addition with weighting factor as a tool for the reliable and efficient quantitation of postmortem toxicology and forensic casework	Danai Taruvinga
P10	Rapid and reliable quantification of six antipsychotic drugs in serum	David Brokaw
P11	Investigation of the cross-reactivity of clonazolam, flualprazolam and flubromazolam with benzodiazepines lateral flow immunoassay tests	Eduardo G. de Campos
P12	A qualitative analysis of THC isomers and derivatives in e-liquids	Emani Smith
P13	Case report: identification of AP-238 and 2-fluorodeschloroketamine in internet available powder samples	Emanuele Alves
P14	Solid phase extraction of novel synthetic 2-benzylbenzimidazole opioid compounds, "Nitazenes"	Emily Eng
P15	A user's history of the mixed bag; designer opiates present in urine samples from heroin users, 2016-2022	Gregory Janis
P16	Quantitative analysis of 10 anticoagulants in human blood by UPLC triple quadrupole mass spectrometry (LCMSMS)	Hiu Yu Lam
P17	The unseen impact of $\Delta 8$ -THC products on the regulated drug testing industry	Jarod Kabulski
P18	Development of an extraction method for the analysis of synthetic opi- oids in bone samples using the Bead Ruptor	Kala Babb
P19	Highly sensitive MS/MS detection for confident identification of potent novel synthetic opioids (NSO) and their metabolites	Karl Oetjen

P20	Conversion of cannabidiol to tetrahydrocannabinol in acidic foods and beverages	Lawrance Mullen
P21	In silico and in vitro phase I biotransformation analysis of synthetic cathinones: eutylone and MPHP	Leonardo B. Maya
P22	Using untargeted high-resolution mass spectrometry and molecular networking for rapid identification of novel psychoactive substances	Maia Bates
P23	Validation of novel synthetic cannabinoid ADB-BUTINACA with LC/MS/ MS	Mary Susan Eley
P24	Methamphetamine and fentanyl use trends in Ohio and Michigan from 2017 to 2021	Matthew P. Levitas
P25	Identification and quantitation of traditional and designer benzodiaze- pines in urine by UHPLC-MS/MS	Michael Clark
P26	Enhancing high-resolution mass spectrometry performance for NPS analysis with improved sensitivity and characterization	Pierre Negri
P27	The rise and fall of nitazene analogs: using standard addition and a uni- form analytical approach to rapidly respond to changes in trends	Rebecca Mastrovito
P28	Comparing performance of LC-QqQ-MS vs. LC-QTOF-MS for targeted analysis of NPS with a test mixture of 40 NPS and metabolites in whole blood, urine, and oral fluid	Rebecca Smith
P29	Identification of multi-year trends of synthetic stimulants detected and identified by GC/MS at the Onondaga County Medical Examiner's Office	Samantha Starkey
P30	Bromazolam concentration profile in Canadian post-mortem cases	Sandrine Merette
P31	Quantitative analysis of designer benzodiazepines in human urine by LC-MS/MS	Shahana W. Huq
P32	Emergence of the nitazene class of novel synthetic opioids in postmor- tem toxicology and detection by LC-QToF-MS	Stuart A.K. Kurtz
P33	Determination of novel psychoactive substances (NPS) and synthetic opioids in meconium	Theresa Lee
P34	Nitazenes at the Roxbury: an analytical workflow for the analysis of emerging synthetic opioids of the nitazene class	Timothy Bolduc
P35	Assessment of recombinant beta-glucuronidase vs native beta-glucu- ronidase and alkaline conditions for the hydrolysis of THC-carboxylated metabolite glucuronide	Alberto Salomone
P36	Raising the quality of initial post-mortem screening to the next level by LC-MS/MS merged product ion scanning	Alexander Giachetti
P37	A fast and green method for LSD urinalysis	Amy Leah Patton
P38	Best practices in urine drug hydrolysis methods	Ana Cabello
P39	Detection of gabapentin in keratinized specimens	Andre Sukta
P40	Evanescent wave technology applied to the detection of drugs in oral fluid	Barry K. Logan
P41	Natural inhibitors in urine can reduce glucuronidase performance and result in lowered recoveries	Claire Collins
P42	Reproducibility of a volumetric absorptive microsampling device for quantitation of drugs of abuse in blood	Colin Bunner
P43	Cutoff concentration evaluation of a fentanyl screening assay	Dan Wang

P44	Withdrawn	
P45	Automated solid phase extraction as-compared-with manual vacuum manifold sample preparation of amphetamines in keratinized matrix analyzed by LC/MS/MS	Dominique Gidron
P46	Solid phase extraction of natural cannabinoids and metabolites from urine and blood	Emily Eng
P47	Analysis of drug-infused papers by ASAP-MS	Emily Lee
P48	Quantitation of urine buprenorphine, norbuprenorphine, and naloxone on an automated platform using room temperature enzymatic hydroly- sis and solid phase extraction	G. Brent Dawson
P49	Direct analysis of drug analytes in complex biological matrices using a molecularly imprinted polymer	Haley Mulder
P50	A chemical profile of commercially available kratom products in Rich- mond, Virginia	James Fleming
P51	The discovery of phenibut in a commercially available product: a case study	James Fleming
P52	Consolidating LC-MS/MS method conditions for the analysis of alcohol metabolites, barbiturates, and drugs of abuse in urine	Jamie York
P53	A one year review of the application of a LC-MS/MS screening method for suspected blood DUI cases	Jazmine Adams
P54	Analysis of benzodiazepines using liquid-chromatography mass spec- trometry-mass spectrometry	Jonathan T. Tomko
P55	When party balloons became taboo: gas chromatograph carrier gas conversion from helium to hydrogen	Joseph Kahl
P56	Assessment of commercially available devices for the removal of hista- mine from red and white wines.	Judith Rodriguez Salas
P57	Comparison of LC-QqQ-MS and DART-QqQ-MS for the detection of drugs in authentic hair specimens	Kaylyn Keith
P58	Sample preparation methods for the analysis of cannabis exposure bio- markers in exhaled breath condensate and oral fluid	Meena Swaminathan
P59	Simultaneous analysis of free steroids and sulfate conjugates by sol- id-phase extraction and LC/MS/MS	Stephanie Reichardt
P60	Testing for drugs of abuse in human blood to ANSI/ASB120 standards by high-resolution, accurate-mass mass spectrometry	Stephanie Samra

POSTER SESSION #2

Thursday, November 3, 12:00-2:00 pm Moderators: Liz Kiely and Steven Raso Exhibit Hall A

Poster Number	Title	Speaker
P61	Distribution of para-fluorofentanyl across multiple matrices in two post- mortem cases	Denice Teem
P62	Performance of LC-Ion Trap-MS screening in forensic toxicology – a sev- en-year recap using proficiency test data of the Toxtyper [®]	Jürgen Kempf
P63	Separation and detection of isomeric cannabinoids and metabolites by LC-MS/MS	Kaitlyn B. Palmquist

P64	The use of isotopically labeled standards in a multipoint internal calibra- tion (MPIC) for the quantification of amphetamine in biological speci- mens by LC-MS	Katherine Gussenhoven
P65	Separation and Quantitation of Natural and Unnatural THC Isomers and Analogues by High-Performance Liquid Chromatography Tandem Mass Spectrometry (HPLC-MS/MS)	Kimberly Karin
P66	Detection of an expanded SAMSHA-7 pre-employment panel by LC-MS/ MS	Kirstin Faoro
P67	Improved THCCOOH isomer separation through alternate column selec- tivity	Larissa K. Karas
P68	Fast derivatized LC-MS/MS Method for separation and quantitation of amphetamine and methamphetamine	Linda Smith
P69	Generation and trapping of reactive drug metabolites by a thiol-con- taining hemoglobin peptide	Ludmyla Santos Tavares
P70	The use of isotopically-labeled standards in a multi-point internal cali- bration (MPIC) for the quantification of methamphetamine in biological specimens by LC-MS	Mackenzie Smith
P71	When life gives you norsertraline, make de-amino-norsertraline!	Madeleine E. Wood
P72	Quantification of phosphatidylethanol in whole blood and dried blood spots by LC-MSMS	Marta Concheiro
P73	Analytical method for the identification and quantitation of 21 cannabi- noids in neat oral fluid by UHPLC-MS/MS	Martin Jacques
P74	An expansion validation of a combined cocaine-opioids method by LC-MS/MS	Megan Savage
P75	The Development of a Virtual Liquid Chromatography Method Develop- ment Tool	Melinda Urich
P76	Identification and quantitation of 13 cannabinoids in whole blood by UHPLC-MS/MS	Melissa Beals
P77	Analytical method for the determination of 15 cannabinoids in urine by UHPLC-MS/MS	Michael Clark
P78	In vitro mitragynine stability	Nancy Kedzierski
P79	Toxicology screening of human blood using quadrupole-time of flight (QTOF) mass spectrometry	Rachel Lieberman
P80	Quantitation of 9 opioids by an automated and fully integrated dried matrix spot module and UHPLC-MS/MS system	Richard Gibson
P81	Applicability of a novel automation platform for column-based drug of abuse extraction prior to LC/MS or GC/MS analysis	Russell Parry
P82	The development of a novel tandem fragmentation technique coupled with trapped ion mobility spectrometry and time of flight mass spec- trometry for the analysis of protein biomarkers indicative of toxicologi- cal threats	Samuel Miller
P83	Cannabinoid Immunalysis ELISA screening in oral fluid samples using Tecan Freedom Evo 75	Sara Jablonski
P84	SAMHSA drug panel screening in oral fluid: development of a rapid screening method using LDTD-MS/MS and Quantisal™ device	Serge Auger

P85	Benzodiazepine drug panel screening in hair: development of a screen- ing method at 8 seconds per sample using LDTD-MS/MS	Serge Auger
P86	Urine drug monitoring: stress testing a new β-glucuronidase enzyme	Vivek Joshi
P87	NIST mass spectral reference libraries	William Brewer
P88	Membrane extraction technology for the analysis of carboxy-THC in urine	William E. Wallace
P89	Characterization of hemoglobin covalent adducts by reactive metabo- lites of cocaine, oxycodone, diazepam, and THC for use as retrospective biomarkers of abused drug exposure	William J. Morrison IV
P90	Discovering CYP2D6 activity biomarkers by correlating MDMA phar- macokinetic parameters to untargeted metabolomics data – a proof of concept study	Yannick Wartmann
P91	The effect of potential interferences on the Dräger DrugTest 5000	Adrienne Chiang
P92	Vaping in schools: a comparative analysis of confiscated vaping devices from public-schools central Virginia from 2019 to 2020	Alaina Holt
P93	RTI's oral fluid proficiency testing program	Amy Evans
P94	The potential to detect 13 cannabinoid acetate analogs in urine using 6 commercially available cannabinoid homogeneous screening kits	Carl Wolf
P95	Evaluation of volatile stability in whole blood containing different addi- tives	Ashley A. Johnson
P96	Baclofen in postmortem casework (2016-2021)	Brianna Peterson
P97	Prevalence of cannabinoids (delta-8 & delta-9 THC and CBD) in urine from a pain management compliance testing laboratory	Carrol Nanco
P98	Towards better understanding SCRAs and metabolites in recreational drug intoxications associated with 5F-MDMB-PICA use	Christophe Stove
P99	Atypical postmortem redistribution in chronic methadone or fentanyl consumers	Corinne Bouchard
P100	Robotrippin' gone wrong – a dextromethorphan fatality on Miami beach	Diane Boland
P101	Analysis of drugs in blood to support the UK Section 5A Driving Under the Influence of Drugs Act	Emily Lee
P102	An unintentional gamma-hydroxybutyrate (GHB) fatality: be careful of the bottle you drink from	Eric S. Lavins
P103	Potential detection of 58 fentanyl analogs in urine using fentanyl immu- noassays	Carl Wolf
P104	Determination of patient adherence for duloxetine in urine	Haley Mulder
P105	Evaluation of multiple oral fluid extractions	Helen Ha
P106	A case study on the acute intoxication of strychnine	Helen Ha
P107	Evaluation of stop-limit practices for DUI samples with BACs > 0.08 g/100 mL	Jarrod Bechard
P108	The quantitation of five antihistamines in blood using LC-MS/MS and the application to human-performance toxicology casework	Kenson Jean
P109	Detection of methamphetamine and metabolite in environmental expo- sure (ChildGuard [®]) cases: a retrospective review	Liaqat A. Abbas

P110	Assessing the effect of COVD-19 on the prevalence of ethanol biomarkers in human urine	Lixia Chen
P111	Multi-method LCMS assay multiplexing with advanced intelligence ca- pabilities to increase laboratory throughput	Logan Miller
P112	Anterograde extrapolation of blood alcohol: comparing Widmark esti- mations to blood alcohol results obtained in a controlled dosing study	Lucien Lockhart
P113	Death by chocolate colored blood – suicidal ingestions of sodium ni- trite/nitrate	Elizabeth Zaney
P114	Analyzing relationships between DRE observations, driving behavior, and THC, methamphetamine, and fentanyl positive toxicology cases in the Michigan Oral Fluid Roadside Analysis Pilot Program	Matthew P. Levitas
P115	Prevalence of designer benzodiazepines in impaired driving casework over a 5-year period (2017-2021)	Michael E. Lamb
P116	Use of the Randox biochip array technology as a screening solution to current drug trends in a clinical setting	Laura Keery
P117	EAGLE FANG: sampling of drug paraphernalia for emerging trends	Sarah Riley
P118	Determination of pharmaceuticals and drugs of abuse in the New York/ New Jersey Harbor estuary water	Teeshavi D. Acosta
P119	Suicidal rocuronium toxicity	Teresa Gray
P120	The incidence of intoxicants in homicide during a surge in homicide deaths	Thomas P. Gilson

S1 - Statistical comparison of optimized and least effective forensic hair analysis methods for multiple drugs and metabolites in authentic user specimens

Brianna Spear, M.S.*, Florida International University, Miami, FL 33199. Anthony P. DeCaprio, Ph.D., Florida International University, International Forensic Science Research Institute, Miami, FL 33199.

Background/Introduction: There are many differing opinions regarding the optimal decontamination, pretreatment, and extraction parameters for hair analysis. Decontamination methods include a variety of aqueous solutions and organic solvents used for removing drugs from the surface of the hair. Pretreatment parameters include incubation times, temperatures, and size of the extracted hair particles. Extraction methods include enzymatic, acid/base, and solvent techniques, each with their own advantages and disadvantages. Previous research in this laboratory utilized the statistical technique known as Statistical Design of Experiments (DoE) to evaluate both the individual roles and the combinatorial associations between multiple variables and drug decontamination/extraction efficiency. These studies utilized authentic HRM, which is prepared by mixing hair from drug users with blank hair to achieve specific drug concentrations, as an effective standard for drug testing, since drug is incorporated into the hair through natural processes of the body. The present study analyzed authentic specimens which were collected from individual drug users and had unknown identities and concentrations of the drugs present.

Objectives: The objective of this work was to statistically compare the optimized and least effective methods previously identified by DoE for analysis of alprazolam, diazepam and nordiazepam, methamphetamine, cocaine and its metabolites, oxycodone, metabolites, of heroin, and fentanyl using authentic specimens.

Methods: Ten authentic user hair specimens were obtained from a collaborating lab (RTI International). Samples of 20 mg each were weighed into 1.8 mL steel milling jars. Specimens processed using the previously identified optimized method were decontaminated with one 30-min wash with HPLC water followed by three 30-min washes with dichloromethane, pulverized into a powder using a Retsch[®] **MM200 ball mill with chrome-steel milling beads at 3,800 rpm for 30 s**, and incubated for 2 h in a 12.5 µL/mg solvent volume/sample weight ratio with methanol:acetonitrile:2 mM ammonium formate solution (25:25:50) at 37°C. Specimens processed using the previously identified least effective method were decontaminated with one 30-s wash with MeOH followed by one 30-s wash with HPLC water, cut into ~1 mm snippets with scissors, and incubated in a 25 µL/mg solvent volume/sample weight ratio with 1 M NaOH at 37°C. All samples were centrifuged in 2 mL Eppendorf tubes for 30 min, prior to solid phase extraction using an Agilent[®] Bond Elut LRC mixed mode C₈ and strong cation-exchange (SCX) cartridge, vacuum centrifugation, and analysis using an Agilent[®] 1290/6460 LC-QqQ-MS with an Agilent 1.8[®] µm Zorbax Eclipse Plus C₁₈ rapid resolution HD column (2.1 x 50 mm; 1.8 µm).

Results: Paired T-Tests were performed post-analysis to determine if the optimized and least effective forensic hair analysis methods resulted in significantly different results. Data indicated that, for the majority of drugs and specimens, the optimized method had significantly higher extraction recoveries than those processed with the least effective method.

Conclusion/Discussion: The results indicate that a consistent, optimized protocol for forensic hair testing for a variety of drugs and metabolites of interest may be possible, providing an opportunity for the improvement of forensic hair testing through standardization of practices and methods.

S2 - Improved automated extraction and high-throughput analysis of Δ9-tetrahydrocannabinol (Δ9-THC) in oral fluid

Omran Muslin1^{*}, Barry Sample², Leslie E. Edinboro^{1,3}, Amanda H. Jones¹, Michael P. Smith¹, and Sarah H. Bartock¹.

¹Quest Diagnostics Nichols Institute, 14225 Newbrook Drive, Chantilly, VA 20151;

²Quest Diagnostics, 101 Renner Blvd, Lenexa, KS 66219;

³Current affiliation: Edinboro Consulting, LLC, 10501 Delray Road, Glen Allen, VA 23060.

Background/Introduction: Novel medicinal and recreational cannabinoid products compel laboratories to differentiate several related cannabinoids. Distinguishing between cannabinoids is critical for monitoring marijuana analytes, as detection of some isomers may have legal implications for patients. Current analytical methods for Δ^9 -tetrahydrocannabinol (Δ^9 -THC) analysis can be susceptible to cannabidiol (CBD) conversion due to acidic conditions in traditional solid phase extraction (SPE) methods.

Objectives: To develop and validate a liquid chromatography tandem mass spectrometry method employing high-throughput multiplexing technology for accurate quantitation of Δ^9 -THC and CBD in Oral Eze^{*} oral fluid using an automated supported liquid extraction (SLE).

Methods: Oral Eze^{*} samples (150 µL) in dilute ammonium hydroxide were mixed with deuterated internal standard in methanol/water, then SLE was performed on Biotage Isolute^{*} SLE+ plate. Analytes were eluted with hexane, dried, and reconstituted in acetonitrile/water. The extraction process was automated with a Hamilton STARlet^{*}. Analytes were then chromatographically resolved by ultra-high performance liquid chromatography (UHPLC) using isocratic separation (water/ammonium acetate/formic acid and methanol/formic acid mobile phases) on a Phenomenex Luna^{*} Omega Polar C18 column (3 µm, 50 x 2.1 mm). Multiplexing reduced total run time from 5 min to 2 min. The assay was validated according to CAP/CLIA guidelines. Limit of detection (LOD), limit of quantification (LOQ), and upper limit of linearity (ULOL) were determined from fortified oral fluid negative buffer. Authentic Oral Eze samples containing high CBD concentrations were subjected to multiple preparation procedures to determine the optimal conditions to prevent CBD conversion to Δ^9 -THC. Specimens were utilized to monitor the rate of conversion in various extraction methods. A comprehensive panel of related cannabinoids was challenged to confirm no significant interferences with Δ^9 -THC quantitation. A within batch interference quality control sample was utilized to achieve analytical confidence and demonstrate a lack of CBD conversion and co-elution of related isomers in each batch.

Results: Isocratic separation of interferences on this column allowed for accurate quantitation of Δ^9 -THC and CBD. For Δ^9 -THC, LOD was 1.0 ng/mL, LOQ was 2.5 ng/mL, and ULOL was 250 ng/mL. For CBD, the LOD was 8 ng/mL, LOQ was 20 ng/mL, and ULOL was 2,000 ng/mL. Previously, under acidic SPE conditions, our experiments demonstrated CBD conversion into Δ^9 - and Δ^8 -THC at a rate of 3% to 5% of the total CBD oral fluid concentration. This conversion was reduced to 1% by removing acidic SPE washes and reduced further to 0.1% by removing acidic SPE loading and washing steps. Conversion was determined from the calculated Δ^9 -THC oncentration divided by the calculated CBD concentration. Certified CBD reference material was verified as Δ^9 -THC-free. Several fortified CBD-only quality controls samples as well as specimens from patients administering certified Δ^9 -THC-free CBD sources were utilized to monitor the rate of conversion in various extraction methods. Changing the sample preparation approach from traditional SPE to SLE achieved the goal of driving CBD conversion in the authentic patients to 0%. Chromatographic separation of Δ^8 and Δ^{10} -THC isomers was also achieved.

Conclusion/Discussion: We developed a validated method for measuring Δ^9 -THC in oral fluid collected with Oral Eze using automated SLE and multiplexed LC-MS/MS instrumentation. To eliminate CBD conversion into Δ^9 -THC, a new extraction method was developed that utilized SLE with a basic sample loading step. This sensitive and specific LC-MS/MS method for cannabinoid detection was validated in oral fluid, which is necessary for workplace, pain management, drug treatment, and forensic drug testing programs.

S3 - Examination of acetone findings in suspected drug-facilitated sexual assault

Crystal Arndt, MSFS, D-ABFT-FT*, Teresa R. Gray, PhD, F-ABFT. Harris County Institute of Forensic Sciences, Houston, TX.

Background/Introduction: Victims of sexual assault frequently undergo forensic medical examinations for the collection of biological evidence in addition to treatment and evaluation. Blood and/or urine samples from the victim may be collected, especially if drug facilitated sexual assault (DFSA) is suspected. At the Harris County Institute of Forensic Sciences, if the investigating agency decides toxicology testing is necessary, the testing scope is dictated by the length of time between the incident and sample collection. Testing for ethanol, acetone, isopropanol, and methanol, as well as other impairing drugs, is performed for specimens collected within 72 hours of the incident. Over the years, we observed that a higher number of DFSA cases have positive acetone findings compared to other human performance cases, such as those for driving under the influence.

While the presence of acetone could be from ingestion, it can also be produced endogenously under certain conditions such as uncontrolled diabetes, fasting, and stress. When a person experiences stress, the adrenal glands release cate-cholamines which escalate the rate of lipolysis causing an increase of glycerol and free fatty acids. The free fatty acids are metabolized to acetyl coenzyme A (CoA) to acetoacetate that further produces acetone and β-hydroxybutyrate.

Objectives: Investigate acetone findings from DFSA cases over a three-year period to aid in the interpretation of toxicological results.

Methods: DFSA cases received from January 1, 2019-December 31, 2021 were reviewed to determine the number and percentage of acetone-positive specimens. Headspace gas chromatography/flame ionization detection (GC/FID) was used to analyze specimens for volatile compounds with a detection limit of 0.010 g/100 mL. Additional drug testing was performed by enzyme linked immunosorbent assay (ELISA), liquid chromatography time of flight mass spectrometry (LC-TOF/MS), and LC/MS/MS.

Results: During the three-year period, DFSA cases comprised 1.4% of the total case load with 393 cases. Of the DFSA cases es submitted, 20% were not tested, 6% were ethanol-positive only, 37% were drug-positive only, 13% were ethanol-and drug-positive, 3% were acetone-positive only, 6% were acetone- and drug-positive, 2% were acetone-, alcohol-, and drug-positive, and 13% had no acetone, alcohol or drugs detected. Overall, 11% of DFSA cases were acetone-positive. For comparison, DWI/DUID cases in that same period numbered 12,007, and only 0.026% had positive acetone results. Acetone was reported qualitatively for 43% of the specimens; in the other 53% of specimens, quantitative values in urine ranged from 0.010-0.147 g/100mL. Of the quantitative results, 71% were 0.010-0.030 g/100 mL, 25% were 0.030-0.090 g/100 mL, and 1 specimen was >0.100 g/100 mL. Acetone was more likely to be detected in urine samples (14%) than in blood (0.8%). When comparing urine samples with positive acetone, alcohol, and/or drug findings, acetone was less likely to be present if other drugs and alcohol were present.

Conclusion/Discussion: Three years of DFSA results were examined to evaluate the prevalence of acetone in blood and urine specimens. The higher prevalence of acetone-positive specimens in DSFA cases may be due to a stress response; however, without full medical history of the victim, other disease states or conditions cannot be ruled out. More research is needed to determine whether acetone has a physiological impact, whether it is a marker for trauma, or if this information is useful for forensic toxicology interpretations.

S4 - DFC casework vs public perception of DFC drugs – fact and fiction?

Meaghan Ringel*, Erin A Spargo, NMS Labs, Horsham, PA.

Background/Introduction: Drug facilitated crimes (DFCs) are defined as the intention of violating the law in conjunction with the presence of a substance to help commit a crime. Examples of DFCs include sexual assault (DFSA), which is the most prevalent, as well as robberies, extortion, and battery. Substances used in these crimes are chosen because of the effects that they produce. Any substance that can compromise the ability of an individual to provide consent, process external stimuli, or create and/or retain memories would be a substance of choice. The media and many universities commonly focus on ethanol, GHB, Rohypnol[®] (flunitrazepam), and ketamine; however, only ethanol is commonly observed in casework.

Objectives: What constitutes a DFC will be defined and the drugs that universities and the media choose to focus on will be examined. Casework submitted to NMS Labs between January 2021 and March 2022 for DFC testing will be evaluated for observed trends. These trends will be compared to what the media portrays as common drugs observed in DFCs.

Methods: DFC bundled testing was performed for 1,586 cases between January 2021 and March 2022: blood/serum and urine - 223 cases, blood/serum only - 205 cases, and urine only - 1,158 cases. Cases were initially screened by liquid chromatography time of flight-mass spectrometry (LC/TOF-MS) for >75 drugs of abuse and therapeutic compounds. Enzyme immunoassay (EIA) screening was performed for barbiturates and cannabinoids and gas chromatography flame ionization detection (GC-FID) was used for generalized volatile screening of alcohols. Confirmation assays were generally performed by liquid chromatography tandem mass spectrometry (LC-MS/MS) or gas chromatography mass spectrometry (GC-MS).

Results: In blood and serum samples, cannabinoids were found to be the most prevalent class of compounds with a positivity rate of 27%, followed by ethanol at 25%, and stimulants (amphetamine, methamphetamine, and benzoylecgonine) at 18%. While benzodiazepines and GHB remain drugs commonly associated with DFCs, they were only observed in 9% and <1%, respectively, of samples. Of the benzodiazepines detected, there were no flunitrazepam or 7-amino flunitrazepam positives. Similar trends were observed in urine samples with cannabinoids, ethanol and stimulants present in 24%, 20%, and 20% of samples, respectively. Again, both benzodiazepines and GHB positive cases were detected, but at much lower percentages (12% and <1%, respectively). Most commonly, cases had no positive reported results for both blood/serum and urine samples (27% and 33% respectively).

Conclusion/Discussion: Cannabinoids is the most prevalent observed drug class in blood/serum and urine samples in DFC cases at NMS Labs, followed by ethanol and stimulants. Both GHB and flunitrazepam, two substances the public and media commonly associate with DFSA cases, had minimal observed positive results. Current drug trends can only be based on reported cases, and it's imperative these cases are reported as quickly as possible. The lack of drug findings in DFCs does not mean a crime did not occur. An individual may be unaware they are a victim of a DFC or may be reluctant to report, causing an increase in the collection interval and an inability to detect some compounds. Additionally, the presence of a drug does not necessarily mean that a substance is directly linked to a crime. The presence of a substance, either therapeutic or recreational, can put an individual at risk for opportunistic DFSA where a perpetrator did not directly administer the victim any drug. Interpretation of the findings should not serve as the sole foundation to prove a crime was committed. Rather, all DFC cases must be accompanied by a thorough investigation.

S5 - Prevalence of xylazine in overdose cases: an analysis of Miami-Dade County Medical Examiner case data

Rocio B. Potoukian*1, Jennifer Gonyea¹, Elisa N. Shoff¹, Marissa J. Finkelstein¹, Anthony P. DeCaprio², George W. Hime¹, Diane M. Boland¹. ¹Miami-Dade Medical Examiner Department Toxicology Laboratory, Miami, FL; ²Department of Chemistry & Biochemistry, Florida International University, Miami, FL.

Background/Introduction: Xylazine is a sedative, muscle relaxant, and analgesic used for animals in a veterinary setting. It is an agonist of α -2 central receptors, which results in the decrease of dopamine and norepinephrine in the central nervous system (CNS). This decrease causes sedation, muscle relaxation, bradycardia, and hypotension. On the street drug market, xylazine can be found in fentanyl and heroin. The combination is referred to as "tranq dope" or "sleep cut" and xylazine on its own is known as "tranq". The dosage used by humans is unclear; research has found that the doses that can produce toxicity and/or death range from 40 mg to 2400 mg. Xylazine is commonly detected using methods of liquid chromatography paired with mass spectroscopy and gas chromatography paired with mass spectroscopy and nitrogen phosphorus detection. Using the Miami-Dade Medical Examiner Toxicology Laboratory's Information Management System (LIMS), case data was gathered for cases between the years 2015-2021 in which xylazine was detected. There are a total of 119 cases being discussed.

Objectives: This presentation will analyze the trends seen in xylazine detection in Miami-Dade and Collier (Naples) counties. Attendees will be able to understand what xylazine is, its dangers, and why it is increasing in popularity.

Methods: Xylazine was extracted from biological matrices using a routine basic drug extraction utilizing solid-phase extraction (SPE). The samples were submitted to a screening method by either liquid chromatography paired with mass spectrometry (LC-IonTrap-MS) or gas chromatography paired with mass spectrometry and nitrogen phosphorus detection (GC-NPD/MS).

Results: An overwhelming number of cases including xylazine are drug overdoses by non-Hispanic white males between the ages of 25-44. There were 30 cases in the 25-34 age range and 30 cases in the 35-44 age range. 82% of all cases were male, and 53% of those males were white. From the total 119 cases, 108 cases (91%) are attributed to polydrug toxicity including cocaine, methamphetamine, and ethanol (Figure 1). Most important to note is that 100% of all cases were fentanyl positive. The fentanyl concentrations ranged from 3.178 to 157.88 ng/mL. Between 2015 and 2021, the amount of xylazine being detected in overdose cases has increased dramatically, with a percent increase of 3350%. From 2018-2021, xylazine was present in 1% of the fentanyl positive cases in 2018, 4% in 2019, 11% in 2020, and 22% in 2021 (Figure 2).




Figure 1: Percentage of other prevalent substances listed in cause of death.

Figure 2: Fentanyl vs. xylazine growth in cases 2015-2021.

Conclusion/Discussion: The prevalence of xylazine in postmortem toxicology casework can represent an upcoming public health crisis. This study aims to bring awareness to the dangers of xylazine as a cutting agent in illicit fentanyl. As the demand for illicit fentanyl increases, it is probable that the amount of xylazine found in postmortem samples will increase.

S6 - Fatal overdose involving the organophosphate insecticide malathion

Stephanie M. Marco, PhD^{1*}, Donna M. Papsun, MS, D-ABFT¹, Luke Kittson², and Aaron Shapiro, PhD, F-ABFT^{3,4,1} NMS Labs, Horsham, Pennsylvania; ² BC Coroners Service, Victoria, British Columbia, Canada;³ BC Provincial Toxicology Centre, Vancouver, British Columbia, Canada;⁴ Department of Pathology and Laboratory Medicine, University of British Columbia, Vancouver, British Columbia, Canada.

Background/Introduction: Organophosphate pesticides (OPs) are widely used in agricultural, residential, and public health pest control applications. OPs inhibit the enzyme acetylcholinesterase (AChE), which inactivates the neurotransmitter acetylcholine (ACh). The subsequent accumulation of ACh at the nerve junction causes the overstimulation of the nervous system. Malathion is an OP that requires bioactivation to malaoxon to inhibit AChE. Although malathion is one of the least toxic OPs commercially available, exposure to large amounts of malathion can be fatal if left untreated.

In this presentation, we discuss a case involving an adult decedent with a history of COPD, cardiomyopathy, and hypertension who was found unresponsive next to a bottle of malathion. Paramedics detected a chemical odor on the decedent and suspected that the decedent had ingested malathion. The decedent was transported to the hospital and treated with atropine and pralidoxime. However, the decedent's disposition did not improve, and death was pronounced approximately 12 hours later. OP was the suspected cause of death. Hospital blood from the decedent was analyzed for cholinesterase (ChE) activity and two malathion metabolites, dimethylthiophosphate (DMTP) and dimethyldithiophosphate (DMDTP). Testing for abused drugs, therapeutics, and alcohol was also performed.

Objectives: This presentation will explore the collaborative decision-making process between death investigators and toxicologists in identifying appropriate toxicology testing and discuss the positive findings. It will also review the toxicology of the OP malathion, including its mechanism of action and the adverse effects of exposure.

Methods: Hospital blood from the decedent was collected in a lavender vial and sent to NMS Labs for analysis. The DMTP and DMDTP quantification in blood was done by standard addition using an existing urine method. Analysis was by LC-MS/ MS with reporting limits of 160 ng/mL (DMTP) and 400 ng/mL (DMDTP). The ChE kit from Roche was used to assess ChE activity according to the manufacturer's instructions. This method involved enzymatic colorimetry with acetylthiocholine as a substrate for ChE. The reporting limit for ChE was 200 IU/L. Abused drugs, therapeutics, and alcohol were analyzed using ELISA, LC/TOF-MS, and Headspace GC.

Results: The toxicology results confirmed that the decedent was exposed to OPs before death. Incidental findings included caffeine and metoprolol.

Whole blood ChE results are typically between the values reported for red blood cells (11188-16698 IU/L) and plasma (1700-5778 IU/L), dependent on factors such as hematocrit. ChE activity is significantly reduced or eliminated in acute OP poisonings. ChE was not detected in the decedent's blood sample, consistent with OP toxicity but not definitive.

Malathion is rapidly metabolized to malaoxon, DMTP, and DMDTP, which are primarily excreted in the urine. Because urine was unavailable for this case, the metabolite concentrations were determined in blood. DMTP and DMDTP were detected at 1600 ng/mL and 2500 ng/mL, respectively. Because these analytes are not routinely analyzed in blood samples, there is limited information to reference for comparison. In individuals occupationally exposed to malathion, DMDTP serum concentrations were reported to range from 100 to 3500 ng/mL. However, the serum to blood ratio of this analyte is unknown.

Conclusion/Discussion: The case history was critical to determining the appropriate toxicology testing for this decedent. Generally, when OP exposure is suspected, blood ChE activity and urine metabolite levels are measured. Given the unavailability of urine and the instability of malathion and malaoxon in blood, the lab tested the hospital blood samples for DMTP and DMDTP. The presence of malathion metabolites in the decedent's blood and the absence of ChE were consistent with OP exposure, the suspected cause of death.

S7 - The relationship between acetone and β-hydroxybutyrate (BHB) Levels in postmortem toxicology casework

Authors: William M. Schroeder II*, Jennifer L. Swatek, Kari M. Midthun; NMS Labs, Horsham, PA.

Introduction: Ketoacidosis is a potentially life-threatening medical condition, where the pH of the blood is reduced due to the build-up of beta-oxidative ketone bodies. Ketoacidosis is commonly the result of complications with diabetes (diabetic ketoacidosis, DKA), alcoholism (alcoholic ketoacidosis, AKA) or diet. Ketone bodies, primarily acetoacetate, acetone, and β-hydroxybutyrate (BHB), are produced in the liver when the body cannot efficiently utilize glucose as an energy source and can result in ketoacidosis. The levels of these ketone bodies in blood may help indicate whether the cause of death is of a diabetic or alcoholic origin or indicative of exogenous ingestion (i.e., acetone ingestion). Routine postmortem toxicology analysis typically includes acetone as part of the scope of testing. However, studies have shown that acetone concentrations alone cannot be the sole indicator for the determination of ketoacidosis. While BHB is a more reliable indicator of ketoacidosis, it is not routinely included in postmortem test panels. Indications of acetone, even below reportable limits, could serve as a marker for deciding whether to add BHB analysis in cases without a clear cause of death. BHB concentrations below 50 mcg/mL are considered normal, as BHB is present endogenously. Concentrations from 51-249 mcg/mL are considered elevated, whereas concentrations >250 mcg/mL are considered high and pathologically significant¹.

Objectives: This presentation will evaluate the relationship between acetone and BHB levels in postmortem forensic toxicology casework performed at NMS Labs from 2019 through 2021.

Methods: Postmortem blood samples were analyzed for the presence of acetone via gas chromatography with a flame ionization detector (GC-FID, Analytical Measurement Range (AMR: 5.0 mg/dL–200 mg/dL) and BHB via gas chromatography - mass spectrometry (GC-MS, AMR: 20 mcg/mL–600 mcg/mL). Cases positive for both compounds were evaluated to see if a relationship between presence and/or concentration(s) can be determined.

Results: Cases analyzed (n = 747) displayed a wide range of results. BHB results ranged from 40-2400 mcg/mL (average 668 mcg/mL). Acetone results for the BHB positive cases ranged from 5.0-200 mg/dL (average 23 mg/dL). Of note, acetone was not always detected above the reporting limit when a significant BHB result was obtained. A poor correlation between acetone and BHB concentrations was observed (R^2 = 0.292). More than 80% of the cases analyzed had BHB concentrations of >250 mcg/mL, indicative of ketoacidosis.

Conclusion/Discussion: Ketoacidosis can be a serious medical condition that has mild symptoms and may have unremarkable findings at autopsy. A reliable toxicological marker of ketoacidosis could be of value in determining a cause of death where one is not easily identified. Acetone provides some limited value in this regard. There appears to be no direct correlation between acetone and BHB concentrations in postmortem blood samples, but the presence of acetone can be a reliable predictor of cases with elevated BHB concentration. Exceptions to this rule do exist, particularly in instances where acetone concentrations are detected below assay reporting limits. Casework has shown that BHB can be present at elevated concentrations without a reportable acetone result. This information, combined with case history, could provide a line of investigation for medical examiners and coroners in determining a cause of death.

1. Elliott et al, Forensic Science International 198 (2010) 53-57

S8 - Mitragynine-only deaths in North Carolina

Justin Brower*, NMS Labs, Horsham, PA.

Introduction: Mitragynine is the primary active alkaloid present in Kratom, the herbal substance prepared from the leaves of the tropical evergreen tree *Mitragyna speciosa*, and it is being seen more frequently in postmortem cases. Pharmacologically, mitragynine is an opioid agonist with an activity of approximately one-fourth that of morphine, but at low doses exhibits stimulant-like properties. Its opioid activity is what fuels its recreational use, but it is also used to combat pain and prevent opioid withdrawal symptoms. Contributing to Kratom's popularity are vocal advocacy groups promoting its use and lobbying to keep it legal in the United States. These organizations often claim that no deaths can be attributed to Kratom because of the presence of other drugs. This is bolstered by the fact that very few mitragynine-only overdose deaths have been reported in the toxicology literature.

Objectives: This presentation will highlight 12 mitragynine-only overdose deaths from the statewide NC Office of the Chief Medical Examiner over a five-year period. These will be compared to similar mitragynine cases containing non-opioid prescription or OTC drugs in therapeutic concentrations and cases involving illicit drugs and opioids. Additionally, postmortem considerations will be discussed when presented with mitragynine-only cases.

Methods: Mitragynine was screened by a validated multi-analyte targeted assay using a high-resolution, accurate mass Thermo Orbitrap LC-MS/MS. Confirmation and quantitation were achieved by either NMS Labs or an in-house validated LC-MS/MS method. Confirmation is not reflexive, and the decision to confirm, and report either qualitatively or quantitatively, was based upon case history and other toxicological findings.

Mitragynine results from a five-year period, from June 2017 through June 2022, were pulled from the toxicology laboratory's LIMS and separated into three bins: qualitative, less than 0.50 mg/L, and greater than or equal to 0.50 mg/L. Considering the pharmacological profile of mitragynine, and knowledge from a previously published analysis of over 1,000 blood specimens, data from the latter bin was used in the investigation of mitragynine-only deaths.

Results: In the five-year period, and from approximately 20,000 cases, the laboratory reported blood mitragynine in 396 cases. The breakdown of those cases was 242 reported qualitatively as "present," 90 less than 0.50 mg/L, and 64 greater than or equal to 0.50 mg/L.

From the 64 cases, 12 were mitragynine-only deaths with no other drugs or alcohol reported, 11 cases contained therapeutic concentrations of non-opioid prescription or OTC drugs, and 41 contained illicit drugs or opioids. The emphasis of this presentation is on the mitragynine-only cases, but a comparison with the other classes will be presented.

HaC	Mitragynine	n	Mean (mg/L)	Range (mg/L)	Frequent other drugs
CH3	Mitragynine	12	2.36	0.73 – 5.9	
N H O CH	+ prescription drugs	11	3.59	1.7 – 6.8	Gabapentin, SSRIs
H ₃ C	+ illicit drugs/opioids	41	1.03	0.50 – 3.8	Fentanyl, Cocaine

Table: Postmortem cases from NC OCME with blood mitragynine concentrations ≥0.50 mg/L.

Conclusion: The idea that Kratom is completely safe because it is natural and has centuries of ethnobotanical use is demonstrably false. Of the twelve mitragynine-only cases presented, all had a cause of death of mitragynine toxicity. In the 11 cases that included non-opioid prescription or OTC drugs, all but one included mitragynine in the cause of death. The remaining 41 cases involving illicit drugs, opioids, or ethanol, were most frequently listed as multi-drug toxicity.

Mitragynine-only fatalities exist, and to avoid missing these causes of death, laboratories should include mitragynine in their screening methods with a plan for confirmation and quantitation.

S9 - Fatal intoxication with o-desmethyltramadol (ODSMT)

Brent Dawson¹, Donald MacNeil², and Laureen Marinetti^{2*}

Redwood Toxicology Laboratory¹, Ascertain Forensics at Redwood Toxicology Laboratory², Santa Rosa, CA.

Background/Introduction: Tramadol is an opioid that is prescribed for its analgesic effects, and O-desmethyltramadol is a pharmacologically active metabolite of tramadol. O-desmethyltramadol is generated by the CYP450 2D6 enzyme whose expression is highly variable among different populations. O-desmethyltramadol has an approximately 200 to 300-fold higher affinity for the mu opioid receptor than that of tramadol and gives rise to the opioid-based adverse drug effects of tramadol. The increased plasma concentrations of O-desmethyltramadol in patients with an ultra-metabolizer phenotype has led some organizations to recommend a 30% reduction of the tramadol dose given to this population group. Even in ultra-metabolizers, tramadol concentrations are typically four times higher than O-desmethyltramadol. This case is the first of its kind in the laboratory in which O-desmethyltramadol is confirmed without the detection of tramadol or N-desmethyltramadol, which suggests ingestion of O-desmethyltramadol. Subsequent review of the death scene, the room of a young male college student, confirmed the presence of a product named ODSMT.

Objectives: The attendee will receive a review of the pharmacology of tramadol. The attendee will be made aware of the availability of O-desmethyltramadol. The ELISA drug screen kit for tramadol has 0.3% cross reactivity for O-desmethyltramadol in both blood and urine, therefore the attendee may want to adjust screening methods if they suspect that O-desmethyltramadol may be used in the demographic that they service. O-desmethyltramadol is a potent opioid capable of being abused resulting in addiction and death.

Methods: The blood sample was screened by headspace GC/FID, ELISA, LCMSMS in both MRM and full scan mode with additional confirmation of O-desmethyltramadol by LC/QTOF-MS. All drugs including O-desmethyltramadol were extracted by either liquid: liquid or solid phase extraction and confirmed and measured using LCMSMS in the targeted mode.

GC/FID	ELISA	LCMSMS
Volatiles - ND	THC-COOH +	O-desmethyltramadol 10900
	Tramadol = ND	Tramadol = ND
		N-desmethyltramadol = ND
		Doxylamine 1130
		Fluoxetine 400
		Norfluoxetine 220
		Delta 8 THC < 2.0
		Delta 8 THC-COOH ~ 96*
		Delta 9 THC – ND
		11-OH-THC - ND
		Delta 9 THC-COOH - ND

Results: Cardiac blood toxicology results in ng/mL were as follows:

ND – not detected * Semi-quantitative method

Conclusion/Discussion: This is the first O-desmethyltramadol case for this laboratory. Internet inquiries revealed that ODSMT is readily available from vendors in the United States, and anecdotal information shows people who prefer to use opioids know about ODSMT. Kronstrand et al. (JAT 2011) described nine unintentional deaths in Sweden caused by a combination of O-desmethyltramadol and mitragynine in an herbal blend. The concentration range of O-desmethyltramadol in the nine cases ranged from 400 to 4300 ng/mL. Tramadol consists of two enantiomers, both of which contribute to analgesic activity via different mechanisms. (+)-Tramadol and the metabolite (+)-O-desmethyl-tramadol are agonists of the mu opioid receptor. (+)-Tramadol inhibits serotonin reuptake and (–)-tramadol inhibits norepinephrine reuptake, enhancing inhibitory effects on pain transmission in the spinal cord. (+)-O-desmethyl-tramadol can also inhibit serotonin

reuptake. Even though fluoxetine was also detected, serotonin syndrome is not likely in this case due to the extremely high concentration of the O-desmethyltramadol. The ingestion of tramadol also produces O-desmethyltramadol but at a lower concentration which likely helps to mitigate and protect the user from too much of the more potent O-desmethyltramadol. This protection is removed when O-desmethyltramadol is the ingested drug. Also of interest in this case is the presence of delta 8 THC and metabolite and the absence of delta 9 THC and metabolites.

S10 - A 5-Year Review of PCP-Related Deaths in Dallas County

Lindsay Glicksberg* and Brittany K. Casey.

Dallas County Southwestern Institute of Forensic Sciences, Dallas, Texas.

Background/Introduction:

Dallas County, in Dallas, Texas, is the second-most populous county within the State of Texas (ninth-most populous in the United States) exceeding 2.6 million people in the 2020 census. The Dallas County Southwestern Institute of Forensic Sciences (SWIFS) serves Dallas County and the surrounding areas through its two divisions – the Office of the Medical Examiner (OME) and the Criminal Investigation Laboratory (CIL). The Toxicology Laboratory within the CIL provides services to identify and quantitate a wide variety of substances and metabolites, including, but not limited to, drugs of abuse, clinical drugs, and alcohol.

Phencyclidine (PCP) is a dissociative anesthetic and has been associated with reckless or dangerous behavior in the user population, as previously reported in the literature. Depending on the circumstances, these types of behavior may result in death. To assess whether these reports were also characteristic of the PCP user population in Dallas County and the surrounding areas, PCP in postmortem casework was evaluated over a five year period.

Objectives: The objective of this study was to evaluate the prevalence of PCP from 2015-2020 in decedents in Dallas County and the surrounding areas in terms of demographical and toxicological information. This evaluation coupled with the medicolegal death investigation narrative, where available, and the cause and manner of death as determined by the Medical Examiner (ME) was used to further assess the role of PCP in potentially fatal behavior.

Methods: Case samples submitted to the Toxicology Laboratory at SWIFS between January 1, 2015 through December 31, 2020 were reviewed for positive-PCP in blood results. Demographical (age, sex, race) and toxicological (PCP concentration, additional/concurrently reported drugs) information was gathered for PCP-positive postmortem cases. Cause and manner of death were also included. Statistical analysis, including Student's T-Test, Single Factor Analysis of Variance (ANOVA), and the non-parametric Mann-Whitney U-test and Kruskal-Wallis tests, were used to evaluate the results.

Results: The Toxicology Laboratory at SWIFS received and analyzed 43,940 cases between January 1, 2015 and December 31, 2020. Of these cases, 898 were positive for PCP, 264 of which were submitted from the OME. Users in medical examiner casework were predominately black (92.1%) males (78.0%) in their late 30s to early 40s (median age: 39 years). Ages ranged from 20 to 71 years old. PCP concentrations ranged from 0.02 - 2.33 mg/L (median: 0.13 mg/L). PCP was the only drug identified in 30.5% of the cases, the remainder had up to nine drugs and/or metabolites present in the sample. The most frequent concurrently reported drug were cannabinoids (39.8%), followed by cocaine and its metabolites (22.0%), and ethanol (18.5%). Manner of death was most frequently ruled "accidental" (61.6%) followed by "homicide" (27.6%). In the accidental deaths, PCP toxicity, alone or with underlying medical conditions, attributed to 25.8% of the deaths; PCP was detected in 43.0% of accidental deaths where cause of death was the result of external forces (e.g., trauma, drowning, smoke inhalation). Gunshot wounds or sharp force trauma was listed as cause of death in homicides cases. Select cases will be presented with behaviors and circumstances stated in the medicolegal death investigation reports.

Conclusion/Discussion: An evaluation of PCP prevalence, details obtained via the investigation narrative where available, and previous literature indicates that PCP use may play a role in behavior characteristic of accidental and homicidal deaths.

S11 - Evaluating drug positivity for Tier I and Tier II drugs relative to BAC thresholds

Amanda L.A. Mohr^{*1}, Melissa F. Fogarty¹, Grace Cieri¹, Barry K. Logan^{1,2}. ¹Center for Forensic Science Research and Education (CFSRE), Willow Grove, PA.

²NMS Labs, Horsham, PA.

Background/Introduction: Impaired driving has been a long-term problem in the United States and is becoming increasingly complex. Drug impaired driving (DUID), novel psychoactive substances (NPS), legalization of recreational drugs, and polysubstance use have further complicated the impaired driving problem taxing laboratory personnel and resources. Per administrative policies, some laboratories may elect not to test for drugs if a blood alcohol concentration (BAC) is above a certain threshold. Stop-limit testing is the practice of making a determination about whether to perform drug testing based on an administratively determined alcohol threshold. In a 2020 survey on drug testing in DUID and traffic fatality cases, 45% (n=64) of laboratories surveyed reported employing a stop-limit testing policy. The limitation associated with this practice is that the contribution of drugs and polysubstance use to the impaired driving problem may be underreported and creates an incomplete data set, hindering the ability to look comprehensively at national DUID data.

Objectives: The objective of this project was to analyze DUID cases for a comprehensive scope, including National Safety Council (NSC) Tier I, Tier II, and NPS and assess the impact of these drug findings relative to various BAC thresholds to assess stop limit testing impacts using a large data set.

Methods: Discarded and deidentified DUID blood samples were analyzed for the study. Two independent liquid-liquid extractions were performed to analyze for basic drugs and for synthetic cannabinoids. All extracts were analyzed using a Sciex TripleTOF® 5600+ LC-QTOF coupled with Shimadzu Nexera UHPLC. A reverse phase gradient of ammonium formate (10 mM, pH 3) and methanol/acetonitrile (50:50) with Phenomenex® Kinetex C18 analytical column was used with the corresponding gradient for the basic and synthetic cannabinoid methods. The basic drug panel library contains over 900 drugs and metabolites, and the synthetic cannabinoid panel contains over 300 drugs and metabolites. Drug positivity was evaluated at >0.08 g/100 mL, >0.10 g/100 mL, which accounted for 75% of the stop-limit levels in the survey, and at >0.15 g/100 mL BAC levels.

Results: A total of 1,426 DUID cases were analyzed, collected between January 2020 and December 2021. Thirty two percent of cases were positive for a Tier I drug only, while 2.6% were positive for a Tier I and Tier II drug. Fifteen percent of cases were positive for ethanol only. Shown in Table 1 are the results for Tier I and Tier II positivity per BAC.

Stop Limit Thresholds					
	All Cases	<0.08 g/100mL	>0.08 g/100mL	>0.10 g/100mL	>0.15 g/100mL
Tier I Only Positivity	32% (n=453)	2.0% (n=29)	9.7% (n=138)	9.0% (n=128)	5.3% (n=76)
Tier II Only Positivity	2.4% (n=35)	0% (n=1)	2.5% (n=36)	2.5% (n=35)	1.5% (n=22)
Tier I and II Positivity	2.6% (n=371)	1.6% (n=24)	4.1% (n=58)	3.4% (n=49)	2.4% (n=34)
Positivity for any Tier I, II or combo	60% (n=859)	3.7% (n=54)	16.2% (n=232)	14.8% (n=212)	9.2% (n=132)

Table 1.	Tier I	and I	l Drug	Positivity	per	BAC
----------	--------	-------	--------	------------	-----	-----

Conclusion/Discussion: Stop limit testing in DUID cases is often justified because there are no enhanced penalties for combined drug and alcohol use, that impairment can be explained by BAC, and limited laboratory resources. Based on the data collected as part of this project, 20% of cases contained ethanol and a drug from Tier I, Tier II or both. Limiting testing based on alcohol results would preclude identification of drug involvement in a number of cases and result in underreporting of drug involvement in impaired driving cases. While it may not be feasible to test for all drugs, testing for at least Tier I drugs in selected impaired driving populations is supported and recommended in order to appropriately assess the extent of drug use by impaired or injured drivers.

S12 - Regional toxicology liaison demonstration project

Chris Heartsill*¹, ***Kristen Burke**¹, ***Sabra Jones**¹, and Amy Miles^{1,2}. ¹Regional Toxicology Liaisons, National Highway Traffic Safety Administration Regions 5, 7, and 9, USA.

²Wisconsin State Laboratory of Hygiene, Madison, WI.

Background/Introduction: There has been a sharp increase in drug-impaired driving across the country. A recent National Highway Traffic Safety Administration report on 2020 Traffic Fatality Data found 38,824 people died on US roadways, with a 6.8% increase in fatal crashes. Of that, 45% of fatal crashes involve risky behavior such as driving impaired by alcohol, speeding, or not wearing a seatbelt. Alarmingly, alcohol-impaired fatalities increased by 14% compared to 2019 data, even with an 11% decline in vehicle miles traveled. The National Safety Council recently published that the traffic death rates in 2021 exceeded the rate of 2019 by 19%.

Objectives: To more fully understand and address the issue of drug-impaired driving, the Regional Toxicology Liaison (RTL) Demonstration Project aims to benefit state toxicology programs through increased support, communications, resources, and criminal justice system coordination; decreased processing time of toxicology samples; and better data reporting. In 2022, the Project established Toxicology Liaisons that support states in NHTSA regions 5, 7, and 9 (https://www.nhtsa.gov/ about-nhtsa), to assist with training, collaboration, and the standardization of testing across state laboratories as well as improving the reporting of data to understand the scope of the drug-impaired driving problem.

Methods: The Regional Toxicology Liaisons work within and collaborate between their respective regions identifying stakeholders within each state, laboratory engagement, collaboration, and evaluation of training requests. The RTLs work together to ensure consistency within the program and share information and resources. Additionally, the Project provides a quarterly report to the SOFT Board of Directors and periodic updates regarding activities, progress, and needs assessments.

Results: Through SOFT, Regional Toxicology Liaisons are involved in various committees to understand current trends in drugged driving, laboratory testing, and laboratory needs. The RTLs are engaged in meetings with stakeholders in each state, including NHTSA regional offices, State Impaired Driving Task Forces, State Traffic Safety Resource Prosecutors, and other regional liaisons, including Judicial Outreach Liaisons and Law Enforcement Liaisons.

Conclusion/Discussion: This presentation will provide an overview of the Project and activities accomplished within the first ten months of the program.

S13 - Rapid extraction and qualitative screening of 30 drugs in oral fluid at concentrations recommended for the investigation of DUID cases

Cynthia Coulter*, Margaux Garnier, Christine Moore.

9-Delta Analytical LLC, Ontario, CA 91761.

Background/Introduction: In 2021 the National Safety Council's Alcohol, Drugs and Impairment (ADID) Division published updated recommendations for drug testing in driving under the influence of drug (DUID) cases which included cutoff concentrations for drugs in blood and oral fluid. Blood analysis is considered the "gold standard" in DUID cases, but generally a warrant and a medical professional are required to collect such an invasive specimen, which involve additional time after a traffic stop, during which drugs are dissipating from the blood. Drugs accumulate in oral fluid by diffusion from the blood, therefore oral fluid reflects the free drug circulating in the body. The main advantage to oral fluid use for DUID cases is the reduced time lapse between traffic stop and specimen collection. Oral fluid collection is easy, non-invasive, doesn't require medical personnel and can be collected at the roadside closer to the time of a suspected impaired driving offense.

Objectives: The purpose was to develop a rapid, simple extraction method followed by qualitative screening using liquid chromatography tandem mass spectrometry (LC-MS/MS) for drugs in oral fluid collected with the Quantisal[™] device. The decision points were selected to be at, or lower, than those recommended as Tier I compounds by ADID for toxicological investigation of DUID cases and also at, or lower, than those recommended by SAMHSA and DOT for Federal workplace drug testing programs. The assay is the first to include all the Tier I drugs and phencyclidine at or below recommended cut-off concentrations.

Methods: A liquid-liquid method using isopropanol, hexane, and ethyl acetate to extract drugs from the oral fluid-buffer mix collected in a Quantisal[™] device, followed by LC-MS/MS was developed and validated according to professional standards. Interference studies, limit of detection, precision at the decision point, ionization suppression/enhancement and processed sample stability were determined.

Results/Discussion: The limit of detection was set at the decision point of the assay and the coefficient of variation for the fifteen replicates at the decision point for all drugs was <4%. Carryover did not occur for any compounds. After 24 hours when compared to a calibration point at the cut-off concentration the processed samples at half the cut-off were still negative, and the extracts at twice the cut-off were still positive. There were no observed interferences from commonly encountered drugs. Ion suppression ranged from to -1.29% for clonazepam to -88% (oxycodone D6). Six compounds showed a small degree of ion enhancement. The earlier eluting compounds generally displayed a higher degree of ion suppression (70%-88%); conversely later eluants displayed <10% ion suppression. The internal standards suitably compensated for matrix effects. As with all screening methods, the result should be confirmed using a validated quantitative assay to ensure confidence in the reported value.

Conclusion: A rapid, sensitive LC-MS/MS method was developed and validated for the screening of Tier I drugs (+ phencyclidine) at, or lower than, the recommended decision points for testing drugs in oral fluid. The assay is the first to specifically target the published drug panel and can easily be implemented routinely for DUID cases as there is no need for additional laboratory instrumentation to be purchased if blood testing is already in place. The method is relatively inexpensive, uses common laboratory instrumentation, has low solvent extraction volumes, and eliminates the need for immunoassay capability.

S14 - Validation of novel cannabinoids including Δ 8-THC, Δ 10-THC, THC-O and THC-P in blood and oral fluid for DUID testing

Kristin Tidwell, D-ABFT*¹, Cody Paseur², Jasmine Maxwell¹, Curt Harper, Ph.D¹. Alabama Department of Forensic Sciences¹, Hoover, Alabama. University of Alabama at Birmingham², Birmingham, Alabama.

Background/Introduction: The passing of the 2018 Agriculture Improvement Act legalized the cultivation and sale of hemp products containing less than $0.3\% \Delta 9$ -THC, the main psychoactive ingredient in marijuana. Since then, novel cannabinoids such as $\Delta 8$ -THC and $\Delta 10$ -THC, which have similar intoxicating effects as $\Delta 9$ -THC, have emerged and are being marketed as "legal highs." Lawmakers in Alabama and other states are attempting to ban or regulate the sale of these products, which has led to an increase in the production of similar cannabinoids. Recently, THC-O-Acetate (THC-O) and Tetrahydrocannabiphorol (THC-P) have emerged as products that are marketed as having an even greater intoxicating effect than $\Delta 9$ -THC. THC-O is reported to be three times as potent as $\Delta 9$ -THC and THC-P is reported to be 30 times as potent.

Objectives: To validate Δ 8-THC, Δ 10-THC, Δ 8-THC-O, Δ 9-THC-O, Δ 8-THC-P, and Δ 9-THC-P in oral fluid and blood and monitor for the presence of these cannabinoids in samples submitted to the Alabama Department of Forensic Sciences in DUID investigations.

Methods: Standards for new cannabinoid targets were purchased and run against existing methods to evaluate for potential interferences. Extraction of cannabinoid targets was performed by liquid-liquid extraction followed by analysis using an Agilent 1260 Infinity Binary Liquid Chromatograph and 6460 or 6470 Triple Quadrupole mass spectrometer with an Agilent Poroshell 120 EC-C18, 2.1 X100 mm, 2.7 micron column. Data analysis was performed using MassHunter Quantiative Analysis Software. Matrix and analyte interference and limit of detection (LOD) studies were conducted per ANSI/ASB Standard 036, Standard Practices for Method Validation in Forensic Toxicology. Interesting case studies will be identified and highlighted.

Results: Under current conditions for both oral fluid and blood confirmation methods, Δ 8-THC, 6aR,9R- Δ 10-THC, 6aR,9S- Δ 10-THC, Δ 8-THC-P and Δ 9-THC-P have adequate separation from each other and from existing cannabinoid targets, but Δ 8-THC-O and Δ 9-THC-O isomers co-elute. Negative matrix samples from Immunalysis Synthetic Oral Fluid, bovine, postmortem, and antemortem blood were analyzed for endogenous interferences. No interferences were noted in any matrix sample. LODs for the blood and oral fluid confirmation methods are below.

Target	Blood (ng/mL)	Oral Fluid (ng/mL)
Δ8-THC	1.0	1.0
6aR,9R-Δ10-THC	2.5	1.0
6aR,9S-∆10-THC	2.5	1.0
Δ8-THC-P	10	2.0
Δ9-THC-P	2.5	1.0
Δ8-THC-O	0.5	1.0
Δ9-THC-O	0.5	1.0

Conclusion/Discussion: With the increase in popularity of novel cannabinoids, it is important to evaluate new targets as they emerge in order to see if there is potential for misidentification with current methods and to ensure that they are not being missed in routine casework. Using current instrument methods for both blood and oral fluid, Δ 8-THC, Δ 10-THC, Δ 8-THC-P and Δ 9-THC-P can be adequately identified, and Δ 8-THC-O and Δ 9-THC-O can be separated from all other cannabinoids except each other. Therefore, THC-O will be reported as non-isomer specific. With the uncertainty of the effects of these products, it is beneficial to monitor routine casework to gather information regarding impact on driving ability. Future aims will be to monitor for the emergence of new cannabinoids.

S15 - Double back - two DUI cases involving lormetazepam, lorazepam, and fentanyl

Stephanie Olofson* Colorado Bureau of Investigation Arvada, CO.

Background/Introduction: Designer benzodiazepines are the most prevalent class of novel psychoactive substances (NPS) drugs detected at the Colorado Bureau of Investigation (CBI). In 2021, Fentanyl jumped to number 5 in the top reported drugs list with increasing concentrations reported. This is a unique case where lormetazepam, lorazepam, fentanyl and norfentanyl were detected in the same individual, stopped twice for suspected Driving Under the Influence (DUI) within 18 hours. Lormetazepam is not approved for use in the United States and can be considered a designer benzodiazepine.

Incident 1: A 57 year old male was stopped at 2312 hours for driving on the curb in a pedestrian only area and running a stop light. Upon being stopped, the individual was unable to place his vehicle in park and turn off the ignition. The driver's eyes were bloodshot, watery, and pupils were constricted. His speech was nonsensical and he appeared unkempt. The officers noted signs of a stimulant as he was restless, talkative, and had an increased sense of alertness; however, also displayed pinpoint pupils common to narcotic analgesics. Horizontal gaze nystagmus (HGN) and lack of convergence (LOC) were present. The walk and turn (WAT) and one leg stand (OLS) were not performed due to safety concerns. Blood was collected at 0023 hours and the driver was released to a sober party.

Incident 2: At 1620 hours, a two vehicle T-bone crash was reported. The driver of the vehicle with front end damage fled the scene, was quickly located, and was the same individual from incident 1. HGN and LOC were not present. Multiple clues were displayed during WAT and OLS maneuvers. For the Modified Rhomberg test, the subject estimated 30 seconds in 20 seconds. The subject was arrested and blood collected at 1722 hours.

Objectives: The goal of this presentation is to describe driving behavior, standardized field sobriety tests (SFSTs) performance and toxicology results for two cases in which the same individual was stopped twice for suspected DUI in under 24 hours with an unique combination of drugs.

Methods: Blood specimens were collected in gray top tubes and submitted to the CBI toxicology laboratory for analysis. Ethanol/volatiles analysis was completed using headspace gas chromatography with flame ionization detection (HS-GC/FID). A 14 panel enzyme-linked immunosorbent assay (ELISA) screened for drugs. Separate benzodiazepine and fentanyl confirmations were performed using solid phase extraction (SPE) with instrumental analysis performed on LC/MS/MS. Lormetazepam is one of 24 different qualitative drugs included in the benzodiazepine and z-drugs assay. The low quality control sample for qualitative benzodiazepines is spiked at 5.0 ng/mL.

Results:		
Drug	Stop 1	Stop 2
Ethanol/Volatiles	Not Detected	Not Detected
Lorazepam	Present less than 5.0 ng/mL	5.7 ng/mL
Lormetazepam	Present	Present
Fentanyl	5.9 ng/mL	3.3 ng/mL
Norfentanyl	3.6 ng/mL	1.6 ng/mL

Conclusion/Discussion: This is a unique case where the combination of lormetazepam, lorazepam, and fentanyl were detected in the same driver suspected of DUI in a 24 hour period. The driver's performance on SFSTs were different at each stop. Lormetazepam has been reported 5 times by the CBI laboratory since 2015.

S16 - Evaluating the practice of triple draws in Colorado with the OSAC guidelines for performing alcohol calculations in forensic toxicology

Vanessa Beall*, Colorado Bureau of Investigation, Arvada, CO.

Background/Introduction: In Colorado, it is standard practice for law enforcement agencies to collect three sequential blood draws, typically taken an hour a part, in felonious or fatal Driving Under the Influence (DUI) investigations. While this practice is not specified by Colorado law, these triple draws have historically been collected to calculate the subject's ethanol elimination rate for a retrograde extrapolation report. The recent OSAC Proposed Standard 2020-S-003 Guide-lines for Performing Alcohol Calculations in Forensic Toxicology states that an elimination rate calculated from two or more test results shall not be used in place of an elimination range.

Objectives: This presentation will examine calculated blood alcohol elimination rates for blood draws taken one hour a part in DUI investigations in Colorado in 2021. The calculated elimination rates will be compared to the range provided in the OSAC guidelines. The presentation will discuss how a high-volume state toxicology laboratory system will need to change their standard of practice to meet the published guidelines.

Methods: The current practice of the Colorado Bureau of Investigation (CBI) toxicology section includes analyzing the three submitted draws for ethanol/volatiles by Headspace Gas Chromatography with a Flame Ionization Detector (HS-GC/FID). Additionally, the CBI has a standardized procedure to perform extrapolations by either applying an elimination range (0.010 - 0.025 g/100mL) for a single draw or a calculated range from the triple draws at the request of law enforcement agencies, prosecutors, or defense attorneys.

Results: In 2021, 8,309 cases were analyzed for ethanol/volatiles by CBI, and 195 of these cases were triple draws (2%). Of the 195 triple draw cases, 142 had ethanol results >0.030 g/100mL for all three blood draws, and the average calculated elimination rate was 0.020 g/100mL/hr. Of the 142 cases, 121 (85%) had a calculated elimination rate within the range recommended by the OSAC guidelines (0.010 – 0.025 g/100mL/hr), 3 cases (2%) had an elimination rate below the recommended range (<0.010 g/100mL/hr), and 18 cases (13%) had an elimination rate above the recommended rage (>0.025 g/100mL/hr). A calculated elimination rate was not determined for cases in which ethanol was not detected (31 cases, 16%), or ethanol was <0.030 g/100mL in the first draw (10 cases, 5%), second draw (4 cases, 2%), or third draw (8 cases, 4%). In 2021, CBI completed 10 retrograde extrapolation reports with 6 (60%) cases using the calculated range.

Conclusion/Discussion: Overall, the practice of determining a calculated elimination rate when conducting a blood alcohol back extrapolation will be evaluated based on the OSAC guidelines and the results of this data analysis. Because the triple draw practice is an established practice in Colorado, considerations for changing blood collection habits, case flow analysis, and customer expectations will be discussed.

S17 - Vaping ethanol: roadside investigations, clinical implications, and subjective effects

Alaina K Holt^{*1,2}, Akansha Anbil³, Madison M Combs³, Megan L Underwood³, Alyssa K Rudy³, Ashlee N Sawyer³, Edward L Boone⁴, Justin L Poklis⁵, Matthew S Halquist⁵, Edgar Greer⁶, Nareg Karaoghlanian⁷, Alison B Breland³, Michelle R Peace¹. ¹Department of Forensic Science. ²Integrative Life Sciences Doctoral Program. ³Department of Psychology. ⁴Department of Statistical Sciences & Operations Research. ⁵Department of Pharmacology & Toxicology, Virginia Commonwealth University, Richmond, VA, United States. ⁶Virginia Commonwealth University Police Department, Richmond, VA, United States. ⁷Maroun Semaan Faculty of Engineering and Architecture, American University of Beirut, Beirut, Lebanon.

Background/Introduction: Electronic cigarette (e-cig) liquid formulations (e-liquids) have been reported to contain as much as 30% ethanol. Ethanol has been demonstrated to be aerosolized by e-cigs, which can then be absorbed across mucosal membranes in the mouth and within deep lung tissue. Anecdotal reports exist describing e-cig users receiving ethanol-positive breath tests and/or other biological test results, though no alcohol was said to be consumed. The Preliminary Breath Test (PBT) and Evidentiary Breath Tests (EBT) are conducted to measure ethanol in exhaled breath, after a 15-20 minute waiting period, and the Standardized Field Sobriety Test (SFST) evaluates several operant conditions to assess ethanol impairment. Potential implications of vaping ethanol could affect a myriad of groups, devices, and settings, including law enforcement officers (LEOs) conducting roadside sobriety tests, ethanol treatment and recovery providers, vehicle interlock systems, workplace drug testing, and court-mandated drug testing.

Objectives: This study evaluated the impact of inhaled ethanol via vaping on the SFST, PBT, and EBT on thirteen participants' using a blind two-by-two experimental design under Virginia Commonwealth University (VCU) IRB HM20015064 and in collaboration with the Virginia Department of Forensic Science, the Center for the Study of Tobacco Products at VCU, VCU Police Department, and City of Richmond Police Department. The study also evaluated the participants' perception of intoxication and impairment with subjective assessment surveys.

Methods: E-liquids were made by a commercial manufacturer at concentrations of 0% and 20% ethanol. Ethanol concentrations were verified in-house using a Shimadzu HS-20 headspace attached to a Nexis GC-2030 gas chromatograph with dual flame ionization detectors. Stability studies were performed monthly to ensure sample suitability prior to clinical use. Thirteen participants were enrolled in the study based on inclusion and exclusion criteria. They vaped either one or ten puffs of an e-liquid (0% or 20% ethanol). LEOs assessed indicators of impairment by following a study design incorporating SFSTs, PBTs, and EBTs at predetermined intervals. Participants answered a computerized survey for self-assessment of impairment.

Results: Ethanol concentrations within the e-liquids were determined to be stable at -20 °C for the duration of the study. Positive breath ethanol results ranged from 0.007-0.030 and 0.013-0.043 g/210 dL by PBT immediately after using a 20% ethanol e-liquid when vaping one puff and ten puffs, respectively. All subsequent PBTs were negative for ethanol. No ethanol or instrument error was detected by EBT for any vaping scenario, at any timepoint. Impairment was not indicated by SFST. Several participants reported feeling some form of intoxication and/or impairment at various points during the study; however, statistically, differences in self-perceived intoxication for the group were not significant.

Discussion/Conclusions: Breath ethanol concentrations were detectable by PBT immediately after vaping an e-liquid containing 20% ethanol. The results demonstrated the standard waiting period of 15-20 minutes employed by LEO before administering a PBT for roadside stops was effective in negating vaping-related false positive breath ethanol results. Vaping ethanol also did not impact SFST results or self-perceived impairment. A well-designed human clinical study with robust and relevant collaborations between a crime lab, police departments, and a research university is important to address pertinent questions to forensic science. Results should be considered when assessing ethanol impairment for e-cigarette users without a waiting period post vaping, as well as functionality of vehicle ignition interlock systems for individuals who use vaping products. Further studies are needed to assess any effects of vaping ethanol on ethanol biomarker concentrations.

S18 - Xanax and driving: an attempted suicide defense case study

Curt Harper, Ph.D*1. ¹Alabama Department of Forensic Sciences, Hoover, Alabama.

Background/Introduction: Alprazolam (Xanax[®]), used to treat anxiety disorders and panic attacks, is the most frequently prescribed benzodiazepine. In 2018, 21 million prescriptions of Xanax were issued to patients. In Alabama, alprazolam was the number one drug found in driving cases in 2014. The typical therapeutic range for alprazolam has been cited at 10-100 ng/mL. However, DUI cases routinely exceed this concentrations range. The crash risk associated with the abuse of benzodiazepines such as alprazolam is 2-10-fold.

Objectives: To investigate the prevalence of alprazolam in DUID cases and highlight an interesting case study involving an attempted suicide defense.

Methods: Blood samples from apprehended drivers suspected of driving under the influence of drugs from 2012-2022 were analyzed for alprazolam. Samples were screened by EIA using a Randox Evidence Analyzer and the Ultra biochip assay. Quantitation was performed by liquid-liquid extraction followed by analysis with an Agilent 6460 Triple Quadrupole LC/ MS/MS. Ethanol was screened by HS/GC/MS and quantitated by a HS/GC dual capillary flame ionization detector.

Results: In 2021, alprazolam was the third most popular drug detected in both DUID arrests and traffic fatalities. Median and average alprazolam blood concentrations in DUID cases were 63 and 88 ng/mL, respectively. Furthermore, when blood alcohol concentrations were 0.01-0.079% and 0.08-0.149%, the prevalence of alprazolam was 22% and 10%, respectively. 95% of drivers were Caucasian with a median age of 35 years old.

Case Study: A 53-year-old Caucasian male drove off the roadway into a residential house killing an elderly disabled woman in her sleep. The subject was disoriented and extremely combative refusing to cooperate with officers and medical personnel. Law enforcement ultimately tased the subject to order to remove him from the vehicle. Toxicological analysis revealed the following findings.

Target	Blood Concentration (ng/mL)	
Ethanol	0.129%	
Benzoylecgonine	160 ng/mL	
Alprazolam	1,400 ng/mL	
Clonazepam	<10 ng/mL	

Retrograde extrapolation and Widmark calculations were performed based on the OSAC Guidelines for Performing Alcohol Calculations in Forensic Toxicology draft document.

Conclusion/Discussion: During courtroom testimony, the defendant admitted to consuming 200 mg of alprazolam (i.e. 200 pills), 80 mg of clonazepam (i.e. 40 pills), and several shots of vodka. He stated that his intention was to commit suicide and he did not have recollection of driving the evening of the crash. Alprazolam at 1,400 ng/mL remains the highest concentration ever in a driving case at ADFS. This is the first known "attempted suicide" defense challenge in a DUI case.

S19 - The effects of COVID-19 on fentanyl concentrations and prevalence in DUID and PM cases in Orange County, CA

Dani C. Mata*1, Vanessa Havro1, Nicholas Casassa1, Kevin Andera1

¹Orange County Crime Lab, Santa Ana, CA.

Background/Introduction: Fentanyl has been triggering national health alerts from the Centers for Disease Control and Prevention since 2015. Since then, it has been detected in both overdose and human-performance cases at increasingly higher frequencies and concentrations. The coronavirus disease 2019 (COVID-19) pandemic changed case submissions for both driver and postmortem (PM) cases which complicated the yearly statistics for fentanyl-related cases. More death investigation cases were submitted for toxicology analysis from pandemic-related deaths and less driving under the influence of drugs (DUID) cases were submitted from reduced drivers on the road.

Objectives: The objective of this presentation is to present four years (2018 – 2021) of fentanyl case information for both PM, bloods and tissue, and human performance blood cases in Orange County, CA during the current COVID-19 pandemic.

Methods: All cases were originally screened using a protein precipitation followed by liquid chromatograph coupled with a quadrupole time-of-flight analysis in blood for 320 drugs, including fentanyl (limit of detection 0.5 ng/mL). The cases were confirmed using a quantitative liquid chromatograph coupled with a tandem mass spectrometer method for 37 drugs, including fentanyl (limit of quantitation 0.5 ng/mL). All concentrations for all matrices were extracted on the confirmation method in duplicate and an average was reported. Both screening and quantitative methods were validated following the ANSI/ASB Standard 036 Standard Practices for Method Validation in Forensic Toxicology. After case completion, toxicological data, demographics, patient history and scene information were compiled. Cases were excluded if there was an indication that fentanyl was administered for a medical reason.

Results: For the four years of this study, the percentage of fentanyl positive cases were 0.9%, 1.4%, 4.0% and 6.5% for drivers from 2018 – 2021. The same data for PM cases was 6.5% 12.4%, 19.0% and 25.8%, respectively. The age range for drivers and death investigation cases was 18 - 83 and 2 - 94 years, respectively, and rarely fluctuated based on year. Each year, males were more than 65% of the fentanyl positive population with drivers in 2019 having the highest male percentage of 82.3%. The fentanyl concentration ranges for drivers over the four years was 0.5 - 303 ng/mL. For death investigation cases, the range of fentanyl in the central blood was 0.7 - 636 ng/mL, in peripheral blood 0.9 - 1100 ng/mL and in brain 1.9 - 441 ng/g. Fentanyl was found in combination with one or more drugs in all but 20 DUID cases and 38 PM cases. Ethanol was the most common co-ingested drugs, followed by methamphetamine, cocaine, benzodiazepines, other opioids and traditional cannabinoids.

Discussion/Conclusion: The pandemic decreased the total number of DUID cases received by the laboratory, yet the percentage of fentanyl DUIDs doubled each year from 2018 – 2021. During the same period, the number of death investigation cases requiring toxicology increased each year, although not to the extent of the DUID cases. With the increase in fentanyl detection and the decrease in morphine, it is not surprising that the traditional opioid and stimulant mixture has changed from morphine to fentanyl with either methamphetamine or cocaine in more than half of DUID cases and more than a third of death investigation cases. Detailed case studies will be presented for both a PM decedent and a DUID incident as it relates to fentanyl concentrations. These cases will enhance the attendees' knowledge of modern-day fentanyl detection and quantitation in a forensic laboratory.

S20 - Impaired driving trends before and during the COVID-19 pandemic in Houston, Texas

Jami Reber*, Jesal Patel, Corissa Rodgers, Peter Stout, and Dayong Lee. Houston Forensic Science Center, Inc., Houston, Texas.

Background/Introduction: The coronavirus pandemic, commonly referred to as COVID-19, largely impacted the United States (US) starting in March 2020, changing life drastically for millions of Americans. In Houston, the fourth largest city in the US, businesses including bars and restaurants were shut down entirely on March 17, 2020, and then slowly reopened with varying capacity limits. Data from impaired driving cases leading up to this shutdown, including previously published trends, can be compared against post-shutdown data to identify any changes in ethanol and drug use during this unprecedented event.

Objectives: This study compared the forensic toxicology results for ethanol and drugs of abuse from impaired driving cases in Houston before and during the COVID-19 pandemic. The number of cases, the distribution of cases with positive blood alcohol concentrations (BACs) and/or drug concentrations, and demographics were evaluated to identify possible differences in the impaired driver population of Houston and trends in drug or alcohol use in this population before and during the COVID-19 pandemic.

Methods: Blood samples from impaired driving cases received by the Houston Forensic Science Center (HFSC) with offense dates between January 01, 2019 and December 31, 2020 were analyzed for ethanol and drugs of abuse. The cases were categorized as pre-COVID (January 01, 2019 through March 16, 2020) and post-COVID (March 17, 2020 through December 31, 2020). BAC results were obtained using headspace gas chromatography with dual flame ionization detectors. The limit of quantitation for ethanol was 0.010g/dL with a linearity range from 0.010-0.500g/dL. Case samples with reported BACs below 0.100g/dL and fatality cases were screened and confirmed for drugs using enzyme-linked immunosorbent assay and gas/liquid chromatography-mass spectrometry.

Results: Over the 2-year period examined, 10,399 blood samples were analyzed by HFSC as part of impaired driving investigations. The mean (median) BAC results for pre- and post-COVID cases were significantly different (ANOVA, p<0.001), with concentrations of 0.137g/dL (0.154g/dL) and 0.130g/dL (0.149g/dL), respectively. Of the age groups evaluated, there was no significant difference in the mean BAC for drivers aged under 21 or over 65 (3% and 2% of cases, respectively) years; however, the mean BAC was significantly lower among drivers aged 21-44 (p=0.037, 74% of cases) and 45-65 (p=0.012, 21% of cases) years. During this time, the percentage of negative ethanol cases increased from 20% pre-COVID to 26% post-COVID. The percent of cases positive only for drugs increased from 17% pre-COVID to 23% post-COVID. The six most prevalent drug classes detected (cannabinoids, benzodiazepines, phencyclidine, cocaine/metabolites, amphetamines, and opioids) saw a slight increase (1-4%) in their percentage of positive cases between pre- and post-COVID offenses. Additionally, there was a higher percentage of polydrug cases, defined as cases confirmed positive for two or more drug classes, in post-COVID samples (16%) versus pre-COVID samples (12%).

Conclusion/Discussion: The observed trends of decreasing ethanol-positive cases and increasing drug and polydrug use among impaired drivers are consistent with previously published HFSC data obtained from 2014-2018 (Rodgers et al. J Anal Toxicol 2021). The previous study saw no significant difference in BAC concentrations between each year examined; however, the present study shows a significant decrease in BAC between both the previously obtained and presently analyzed data, as well as between the pre- and post-COVID results. Several studies similarly observed an increase in drug use with varying trends of ethanol use during the COVID-19 pandemic compared to previous years (Webster et al. CDAR 2021; Vanlaar et al. Accid Anal Prev 2021; Thomas et al. NHTSA 2020). Continuing to monitor trends in impaired driving can help establish better enforcement and preventative measures to identify and reduce future impaired driving instances.

S21 - First identification, chemical analysis, and pharmacological characterization of *N*-piperidinyl etonitazene (etonitazepipne), a recent addition to the 2-benzylbenzimidazole opioid subclass

Marthe M. Vandeputte^{1,*}, Nick Verougstraete^{1,2}, Donna Walther³, Grant C. Glatfelter³, Jeroen Malfliet⁴, Michael H. Baumann³, Alain G. Verstraete^{2,5}, Christophe P. Stove^{1,*}.¹ Laboratory of Toxicology, Department of Bioanalysis, Faculty of Pharmaceutical Sciences, Ghent University, Ghent, Belgium;² Department of Laboratory Medicine, Ghent University Hospital, Ghent, Belgium;³ Designer Drug Research Unit, Intramural Research Program, National Institute on Drug Abuse, National Institutes of Health, Baltimore, MD;⁴ Huisartsenpraktijk De Leeuwerik, Dendermonde, Belgium; ⁵ Department of Diagnostic Sciences, Ghent University, Ghent, Belgium.

Background/Introduction: The number of new synthetic opioids (NSOs) appearing on the recreational drug market continues to increase. While newly emerging opioids present with progressively diverse chemical structures, opioids with a 2-benzylbenzimidazole core ('nitazenes', e.g. isotonitazene, etodesnitazene) have recently become increasingly prevalent.

Objectives: *N*-piperidinyl etonitazene ('etonitazepipne') represents one of the most recent additions to the rapidly expanding class of 2-benzylbenzimidazole 'nitazene' opioids. Following its first identification in an online-sourced powder and in biological samples from a patient seeking help for detoxification, this report details its in-depth chemical analysis and pharmacological characterization (with a focus on the latter).

Methods: An online-sourced powder containing *N*-piperidinyl etonitazene was analyzed via LC-HRMS (full scan range of 70-1000 *m/z* using data-dependent acquisition), GC-MS, UHPLC-DAD and FT-IR. Furthermore, an activity-based bioassay (monitoring activation of the μ -opioid receptor (MOR)) was used to detect *N*-piperidinyl etonitazene in serum and urine. Molecular networking of obtained HRMS data enabled the tentative identification of urinary metabolites. *In vitro* characterization encompassed radioligand binding assays in rat brain tissue, and a MOR- β -arrestin2 activation assay. The pharmacodynamic effects induced by *N*-piperidinyl etonitazene in male Sprague Dawley rats included assessment of antinociceptive, cataleptic, and thermic effects.

Results: Analysis of the powder via different techniques led to the unequivocal identification of *N*-piperidinyl etonitazene. Furthermore, the first activity-based detection and analytical identification of *N*-piperidinyl etonitazene in authentic samples was reported. LC-HRMS analysis revealed concentrations of 1.21 ng/mL in serum and 0.51 ng/mL in urine, whereas molecular networking enabled the tentative identification of various (potentially active) urinary metabolites. In addition, the use of an activity-based bioassay indicated that the extent of opioid activity present in the patient's serum was equivalent to the *in vitro* opioid activity exerted by 2.5-10 ng/mL fentanyl or 10-25 ng/mL hydromorphone in serum. Radioligand binding assays in rat brain tissue indicated that the drug binds to MOR with high affinity (K_i=14.3 nM). Using a MOR- β -arrestin2 activation assay, it was shown that *N*-piperidinyl etonitazene is highly potent (EC₅₀=2.49 nM) and efficacious (E_{max}=183% *versus* hydromorphone) *in vitro*. Pharmacodynamic evaluation in rats showed that *N*-piperidinyl etonitazene induces opioid-like antinociceptive, cataleptic, and thermic effects, its potency in the hot plate assay (ED₅₀=0.0205 mg/kg) being comparable to that of fentanyl (ED₅₀=0.0209 mg/kg), and >190 times higher than that of morphine (ED₅₀=3.940 mg/kg).

Conclusion/Discussion: *N*-piperidinyl etonitazene ('etonitazepipne') is one of the most recent synthetic opioids to emerge on recreational drug markets in the U.S. and Europe. With its characteristic 2-benzylbenzimidazole core structure, the drug represents one of the latest additions to the increasingly widespread group of 'nitazene' opioids. This study presents the first identification, chemical analysis, and pharmacological characterization of *N*-piperidinyl etonitazene. The drug was identified for the first time in an online-sourced powder and in the serum and urine of a patient. Various *N*-piperidinyl etonitazene metabolites were tentatively identified in urine. Furthermore, *in vitro* and *in vivo* findings demonstrated that *N*-piperidinyl etonitazene is a potent MOR agonist. Taken together, these data indicate that misuse of *N*-piperidinyl etonitazene (etonitazepipne) is of great concern to public health.

S22 - Evaluating cross reactivity of new psychoactive substances (NPS) on immunoassay in whole blood

Grace Cieri*¹, Amanda LA Mohr¹, Rebecca Mastrovito², and Barry K Logan^{1,2}, ¹Center for Forensic Science Research and Education, Fredric Rieders Family Foundation, 2300 Stratford Ave, Willow Grove, PA ²NMS Labs, 200 Welsh Rd, Horsham, PA.

Background/Introduction: The use of new psychoactive substances (NPS) has been increasing since 2010 when they were first identified. In 2020, the United Nations Office of Drugs and Crime reported a total of 950 NPS which increased to over 1,100 less than a year later in November of 2021. Because of the rapid emergence, there is often little knowledge about the adverse effects or published methods on detecting NPS in biological matrices. Traditional screening in toxicology laboratories is often performed using enzyme linked immunosorbent assays (ELISA). While commercial kits provide information on cross-reactivity on other drugs within a class, many have limited or no information on emerging NPS.

Objectives: The objective of this presentation is to describe the cross reactivity of NPS opioids, NPS stimulants, NPS benzodiazepines and NPS hallucinogens on commercially available immunoassay kits for the purpose of toxicological screening.

Methods: Neogen fentanyl, opiate, amphetamine, benzodiazepine, and PCP ELISA kits were purchased for the project. Fifty different drugs across the various NPS subclasses were analyzed. Blank human whole blood was fortified with the target drug initially at concentrations below the cutoff of the ELISA plate. If no cross reactivity was observed, concentrations above the cutoff were evaluated. Cutoff concentrations were as follows: fentanyl 0.2 ng/mL, morphine 50 ng/mL, amphetamine 20 ng/mL, oxazepam 40 ng/mL and PCP 10 ng/mL. All fortified blood samples, controls and blanks were analyzed on the ELISA plates in duplicate. Following the addition of the fortified samples and drug conjugate, samples were run according to the manufacturer's instructions using Titertek-Berthold Crocodile Mini Workstation. Optical densities were collected at 450 nm. The average optical density was plotted relative to concentrations to assess cross-reactivity and percent cross-reactivity was calculated.

Results: Novel synthetic opioids were tested at concentration ranges of 1-80 ng/mL and 50-2000 ng/mL and had no cross-reactivity to the morphine ELISA plate at either concentration range. Fentanyl analogs were tested at concentrations ranging from 0.01-1 ng/mL on the fentanyl plate and had cross-reactivities ranging from 8.3%-178%. Para-chlorofentanyl, acryl fentanyl, furanyl fentanyl, para-bromofentanyl, and ortho-fluorofentanyl all had cross-reactivities greater than 100%. Para-fluorofentanyl had a cross-reactivity of 84%. NPS stimulants were tested at concentration ranges of 0.5-40 ng/mL and 20-2000 ng/mL on the amphetamine plate. 4-fluoroamphetamine was the only drug with cross-reactivity (3,354%). Prevalent NPS stimulants such as eutylone showed no cross-reactivity. NPS benzodiazepines were tested on the oxazepam plate at concentration ranges of 1-40 ng/mL and 40-500 ng/mL. Cross-reactivities greater than 100%. NPS hallucinogens were evaluated on the PCP kit at concentration ranges of 0.5-10 ng/mL, which showed no cross-reactivity, and 10-1000 ng/mL, where some cross reactivity was observed. Hydroxy-PCP and chloro-PCP both had cross-reactivities greater than 100%.

Conclusion/Discussion: Following the cross-reactivity assessment, the structures of the NPS evaluated were compared to the target drug for each kit, which, in general, indicated that the more closely the structure aligned with the target drug, the better the cross reactivity. NPS benzodiazepines and fentanyl analogs, which are seen with limited frequency in cases since core structure scheduling, showed the greatest cross-reactivity across the class demonstrating the utility of using ELI-SA-based screening for these NPS subclasses. However, limited cross-reactivity was observed for the other NPS subclasses. Therefore, the utility of ELISA-based screening NPS subclasses is limited, and there is the risk of false negative results due to the low or nonexistent cross-reactivities.

S23 - Red herrings, white lies, and Blue Lotus

Joshua Z. Seither*, Erin L. Karschner, and Jeffrey P. Walterscheid

Division of Forensic Toxicology, Armed Forces Medical Examiner System, Dover AFB, DE, USA.

Background/Introduction: Blue lotus commonly refers to *Nymphea caerulea*, an aqueous lily plant that has been recently found in products that reduce anxiety and aid in sleep. The active components of blue lotus have been reported to be two aporphine alkaloids, nuciferine and apomorphine. These compounds are being explored for medicinal purposes and currently have very little forensic toxicological significance. As with other novel psychoactive substances, illicit drug manufacturers and suppliers continue to attempt to circumvent scrutiny by using innocuous names such as "herbal incense" or "potpourri" when branding synthetic cannabinoids. This potentially allows the sale of these products in head shops or online websites. It appears that "blue lotus" is still being used for synthetic cannabinoid offerings. This may hinder forensic investigations, as law enforcement officers or forensic investigators may overlook these products or request incorrect testing from toxicology laboratories.

Objectives: After attending this presentation, participants will be aware of "blue lotus" products and how to properly evaluate toxicology specimens to reveal the underlying intoxicating substances. In addition, toxicological findings from authentic cases where "blue lotus" use was mentioned in case history or "blue lotus" testing was requested will be presented.

Methods: Specimens submitted specifically for "blue lotus" testing, or where "vaping blue lotus" was mentioned in case history were screened by a liquid chromatography quadrupole time of flight mass spectrometry (LC-QTOF/MS) method designed specifically for synthetic cannabinoids. Positive screening results were subsequently confirmed by a LC-tandem mass spectrometry (LC-MS/MS) method with limits of detection ranging between 0.1 and 1.0 ng/mL in blood or urine. Both analytical methods were validated according to ASB Standard 036 guidelines.

Results: From May 2020 to May 2022, synthetic cannabinoids were confirmed in 29 "blue lotus" cases. Results showed 17 cases of 5F-MDMB-PICA, 8 cases of MDMB-4en-PINACA, 5 cases of 4F-MDMB-BUTICA, 4 cases of ADB-BUTINACA, 3 cases of 4F-MDMB-BUTINACA 3,3-dimethylbutanoic acid, 1 case of MMB-FUBICA, and 1 instance of 5F-PB-22 3-carboxy-indole. There were eight cases where more than one distinct synthetic cannabinoid was confirmed. In addition, direct analysis of "blue lotus" vaping solution retrieved from at least one investigation scene also confirmed the presence of 5F-MDMB-PICA.

Conclusion/Discussion: "Blue lotus" appears to be a misleading term to advertise synthetic cannabinoids as a healthy, natural, herbal substitute for nicotine vaping. Most of the toxicology specimens analyzed support 5F-MDMB-PICA as the primary compound found in "blue lotus"-related casework; however, other synthetic cannabinoids may be present. Since little information is known regarding nuciferine and apomorphine, confusion could inadvertently steer toxicology analyses towards those alkaloids. Instead, it is recommended that any mention of "blue lotus" or description of unexplained intoxication related to vaping be sent for synthetic cannabinoid analysis. These results demonstrate how important it is to stay abreast of current trends, frequently update methods with relevant synthetic cannabinoid compounds, and conduct synthetic cannabinoid analyses as part of routine forensic toxicology casework.

S24 - Designer benzodiazepine trends in Northeast Ohio: November 2019 - March 2022

Kimberly Yacoub^{*1}, Christie Mitchell-Mata¹, Luigino Apollonio¹. ¹Cuyahoga County Regional Forensic Science Laboratory, 11001 Cedar Ave, Cleveland, OH 44106. *Presenting author

Background/Introduction: Benzodiazepines are used to treat neurological and psychiatric disorders, but the abuse of these drugs is rapidly growing. Not only are prescription benzodiazepines being abused, but also designer benzodiazepines (DBZD), a class of novel psychoactive substances (NPS), similar in property to prescription benzodiazepines. The abuse of DBZD is concerning as these drugs are produced in unregulated labs, purchased on the dark web without oversight and currently, there are no approved uses for them in the United States. Although the presence of certain DBZD is constantly changing, it is important to detect these compounds and understand their impact in forensic investigations.

Objectives: This presentation will highlight trends of postmortem and antemortem DBZD cases in blood and urine throughout Cuyahoga County and surrounding areas between November 2019 and March 2022. The six DBZD targeted for study include etizolam, flualprazolam, bromazolam, clonazolam, clobazam and flubromazolam.

Methods: Between November 2019 (Q4) and December 2020, 4,045 total cases were submitted to the Cuyahoga County Regional Forensic Science Lab (CCRFSL), while between January 2021 and March 2022 (Q1), 3,972 total cases were submitted. These include postmortem, human performance, and drug-facilitated sexual assault cases throughout Cuyahoga County and neighboring counties. For benzodiazepine, cases are screened using enzyme-linked immunosorbent assay (ELISA) with confirmatory testing by liquid chromatography-mass spectrometry (LC-MS/MS). An in-house LC-MS/MS method was developed to detect 40 benzodiazepines including metabolites and Z-drugs in November 2019¹. With limits of detection that range between 0.5 ng/mL to 10 ng/mL, the 19 DBZD and metabolites include clonazolam, deschloro-etizolam, etizolam, flubromazolam, flubromazepam, clobazam, estazolam, phenazepam, meclonazepam, methyl-clonazepam, normazolam, nitrazolam, flunitrazolam, 2-Hydroxyethylflurazepam, methyl-clonazepam, and N-desmethylclobazam. Of these 19 compounds, six were found most frequently in casework: etizolam, flualprazolam, clobazam and flubromazolam.

Results: During the span of this 2.5-year period, the most frequently detected DBZD was etizolam, closely followed by flualprazolam with an occurrence of 44 and 34 cases, respectively. The incidence of etizolam cases remained near 30% between the two time periods, and the incidence of flualprazolam cases decreased from 32% to 19%. In 2021/Q1 2022, there was a notable appearance of bromazolam (15 cases) that was not seen in Q4 2019/2020. Clonazolam, clobazam and its metabolite, and flubromazolam were seen at a similar occurrence between the two time periods, approximately 16%, 3%, and 8%, respectively. Pyrazolam was detected in one case between Q4 2019/2020 compared to zero pyrazolam cases detected in 2021/Q1 2022.

Conclusion/Discussion: Routine testing of NPS drugs like DBZD is relevant for toxicological casework. Similar to what was observed on a national level at The Center for Forensic Science and Research (CFSRE), etizolam was detected at the highest rate followed by flualprazolam and clonazolam². In the ever-changing DBZD landscape, continually adding or removing NPS compounds to methods is necessary. Recently, based on the CFSRE and Society of Forensic Toxicologists NPS Committee scope recommendations³, an additional 10 compounds have been added to the current in-house LC-MS/MS benzodiazepine method for qualitative reporting in order to monitor the prevalence of newer DBZD.

References:

- 1. Sofalvi, S., Lavins, E.S., Kaspar, C.L., Michel, H.M., Mitchell-Mata, CL., Huestis, M.A., Apollonio, L.G. Development and Validation of an LC-MS-MS Method for the Detection of 40 Benzodiazepines and Three Z-Drugs in Blood and Urine by Solid-Phase Extraction. *Journal of Analytical Toxicology*. 2020 Oct 12; 44(7): 708-717.
- Krotulski, A.J., Walton S.E., Mohr A.L.A. and Logan B.K. (2022) Trend Report: Q1 2022- NPS Benzodiazepines in the United States. <u>https://www.npsdiscovery.org/wp-content/uploads/2022/04/2022-Q1_NPS-Benzodiazepines_</u> <u>Trend-Report.pdf</u>
- Krotulski, A., Papsun, D., Mata, D., Ellefsen, K., Shoff E., Shanks, K., Sofalvi, S., Lewallen, W., Vikingsson, S., Logan, B. (2022) Recommended Scope for NPS Testing in the United States Q1 2022. <u>https://www.npsdiscovery.org/re-ports/nps-scope-recommendations/</u>

S25 - Quantitation of the new synthetic cathinone N,N-Dimethylpentylone in a post-mortem case series

Melissa F. Fogarty^{1,*}, Sara E. Walton¹, Donna M. Papsun², Michael Lamb², Barry K. Logan^{1,2}, Alex J. Krotulski¹. ¹Center for Forensic Science Research and Education (CFSRE) at the Fredric Rieder's Family Foundation, Willow Grove, PA, USA;²NMS Labs, Horsham, PA, USA.

Background/Introduction: Synthetic cathinones emerged over ten years ago onto the novel psychoactive substances (NPS) drug market as substitutes for "ecstasy," "Molly", and "MDMA". Following similar patterns of other subclasses of NPS, these drugs have exhibited rapid turnover in positivity post-scheduling of specific drugs at the international level. The most recent example is the decline in eutylone positivity after the recommendation of international control in September 2021, which subsequently led to the emergence of *N*,*N*-dimethylpentylone. *N*,*N*-Dimethylpentylone, an isomer of *N*-ethyl pentylone, is a new synthetic cathinone not previously identified in the recreational drug supply; therefore, toxicology labs lacked the ability to detect and quantify this drug.

Objectives: The purpose of this presentation is to describe an analytical method capable of chromatographically resolving the isomeric beta-keto methylenedioxyamphetamines (bk-MDAs) related to *N*,*N*-dimethylpentylone and showcase the concentrations of *N*,*N*-dimethylpentylone found in authentic forensic toxicology specimens.

Methods: The following analytes were included in this method: isomers *N*,*N*-dimethylpentylone, *N*-ethyl pentylone, diethylone, hexylone, and tertylone, and isomers pentylone, and eutylone. Biological specimens (0.5 mL) were extracted using a basic (pH 10.4) liquid-liquid extraction. Samples were diluted, as necessary, to fit into the calibration range based on previous analyses (e.g., screen via LC-QTOF-MS).

Quantitative analysis was performed using a Waters Xevo TQ-S Micro tandem mass spectrometer coupled with a Waters Acquity UPLC[®] (Milford, MA). Chromatographic separation was achieved using an Agilent Poroshell EC C-18 column (3.0 mm x 100 mm, 2.7 μ m) (Santa Clara, CA) heated to 60°C with a flow rate of 0.4 mL/min. The mobile phases were 5 mM ammonium formate in water, pH 3 (A) and 0.1% formic acid in acetonitrile (B) used in the following 7 min gradient: 90A:10B, increasing over 5.5 mins to 65A:35B, increasing again to 95%B over 0.5 mins, and at 6.1 mins returning to initial conditions with a 0.9-minute hold.

Authentic specimens were initially quantified via standard addition (up-spikes at 0, 1, 10, and 100 ng/mL) to determine the appropriate concentration range for method validation. As the number of submitted cases increased, a fit-for-purpose method validation was performed evaluating the following criteria: accuracy, intra- and inter-precision, linearity, limit of detection, limit of quantitation, and matrix and commonly encountered interferences. Dilution integrity was performed in blood at 1:2 and 1:10 and in urine at 1:10 and 1:50.

Results: All bk-MDAs met acceptability criteria during the validation experiments. All calibration curves were calculated using a quadratic fit with 1/x weighting regression and a resulting calibration range of 10-1000 ng/mL. All drugs met acceptability requirements of accuracy (<±20% from target) and intra- and inter-precision (coefficient of variation <±15%).

Forensic investigations suspected to involve *N*,*N*-dimethylpentylone were submitted to our laboratory starting in September 2021, primarily from Florida or New York. To date, *N*,*N*-dimethylpentylone has been quantitated in 18 postmortem cases with a resulting concentration range of 3.3 to 970 ng/mL (median: 145 ng/mL, mean 277±283 ng/mL). Pentylone, a suspected metabolite of *N*,*N*-dimethylpentylone, was detected in all cases (range: 1.3-420 ng/mL, median: 31 ng/mL, and mean: 88±127 ng/mL). *N*,*N*-Dimethylpentylone was found in combination with fentanyl (n=7), eutylone (n=6), and methamphetamine/amphetamine (n=5). An additional 62 cases are pending analysis as the prevalence of *N*,*N*-dimethylpentylone continues to increase.

Conclusion/Discussion: Blood concentrations reported for *N*,*N*-dimethylpentylone follow similar trends of previously reported synthetic cathinones (e.g., eutylone). In all cases, the concentration of pentylone was less than *N*,*N*-dimethylpentylone, further supporting that pentylone is a metabolite, however, co-ingestion cannot be ruled out. With this sudden increase in identifications of *N*,*N*-dimethylpentylone, if forensic toxicologists find samples positive for pentylone it is recommended that they be further investigated for *N*,*N*-dimethylpentylone at this time with methodology that separates N,N-dimethylpentylone from its isomer *N*-ethyl pentylone.

S26 - "Diet-Weed's" (Δ8-THC) cross-reactivity with six commercial cannabinoid urine screening kits

Ashley A. Pokhai^{*1}, Justin L. Poklis², Grace R. Williams³, and Carl E. Wolf^{1,3}. ¹ Department of Forensic Science. ² Department of Pharmacology & Toxicology. ³ Department of Pathology, Virginia Commonwealth University, Richmond, VA.

Background/Introduction: Following legalization of hemp (Agricultural Improvement Act of 2018), there has been an increased presence and use of cannabinoids. This growth is not only attributed to Δ 9-tetrahydrocannabinol (Δ 9-THC) and cannabidiol (CBD), the most abundant phytocannabinoid components of cannabis and hemp, respectively, but also other THC analogs. Recently, Δ 8-tetrahydrocannabinol (Δ 8-THC) has become readily available for commercial use in various products including e-cigarettes, edibles and powders. Currently, Δ 8-THC is considered legal; however, with similar potency to Δ 9-THC, it has the potential for abuse. Δ 8-THC, a positional isomer of Δ 9-THC, is metabolized to the carboxylic acid (Δ 8-COOH-THC) and 11-OH- Δ 8-THC. Consequently, its detection may be amenable to current immunoassays used for the detection of Δ 9-COOH-THC in urine. The federal cannabinoid screening cutoff is 50ng/mL, however 20 or 25ng/mL are used to further extend the detection window in certain cases.

Objectives: To evaluate the ability of six homogeneous urine cannabinoid screening kits to detect Δ 8-THC and its biological urine metabolites at 20 or 25ng/mL and 50ng/mL cutoff concentrations.

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

Results: Not all analogs were detectable by each immunoassay. For detectable analogs, the minimum detectable concentration was 50ng/mL. Δ 8-THC was detected at 100ng/mL using the lower cutoff (20 or 25ng/mL) by Abbott(20), DRI(20), LZI(25), and SYVA(20). Δ 8-THC was detected at 200ng/mL using the federal cutoff (50ng/mL) by Abbott, DRI, LZI, and SYVA. 11-OH- Δ 8-THC and Δ 8-COOH-THC were detected at 50ng/mL using the lower cutoff (20 or 25ng/mL) by Abbott(20), CE-DIA(25), DRI(20), LZI(25), ROCHE(20), and SYVA(20). 11-OH- Δ 8-THC and Δ 8-COOH-THC were detected at 100ng/mL using the federal cutoff (50ng/mL) by Abbott, DRI, LZI, and SYVA. 20). 11-OH- Δ 8-THC and Δ 8-COOH-THC were detected at 100ng/mL using the federal cutoff (50ng/mL) by Abbott, CEDIA, DRI, LZI, ROCHE, and SYVA. Olivetol was detected at 1000ng/mL by ROCHE at both cutoff concentrations. Olivetol-COOH was not detected by any immunoassay.

Conclusion/Discussion: 11-OH- Δ 8-THC and Δ 8-COOH-THC were detectable by all assays at each cutoff at approximately two times the Δ 9-COOH-THC cutoff concentration. Δ 8-THC was only detected by the Abbott, DRI, LZI and Syva assays at both cutoffs at approximately four times the Δ 9-COOH-THC cutoff concentration. Olivetol was detected by the ROCHE assay at a large concentration. Olivetol-COOH was not detected.

S27 - Benzo-dope: an increasingly prevalent drug combination of significant toxicological relevance

Alex J. Krotulski^{*1}, Donna M. Papsun², Sara E. Walton¹, Barry K. Logan^{1,2}. ¹Center for Forensic Science Research and Education, Fredric Rieders Family Foundation, Willow Grove, PA. ²NMS Labs, Horsham, PA.

Background/Introduction: Drug overdose deaths continue to increase year after year in the United States as the Centers for Disease Control and Prevention reported more than 100,000 overdose deaths in 2021. These deaths are largely fueled by opioids and stimulants; however, the drug overdose crisis has entered a new wave marked by poly-drug formulations and usage. The prevalence of fentanyl continues to increase across the United States since its most recent introduction into the drug supply in the late 2000s. Today, most regions of the country see fentanyl more commonly than heroin and other opioids. As fentanyl has gained a strong hold on the opioid supply, manufacturers of the drug products continue to seek a product with the "next best high". This has led to the recent emergence of several fentanyl drug combinations, including SCRA-dope (fentanyl with synthetic cannabinoids), tranq-dope (fentanyl with xylazine), and benzo-dope (fentanyl with benzodiazepines). Benzo-dope poses significant health risks due to the combined pharmacological effects of the two CNS depressants.

Objectives: The primary objectives of this presentation are 1) to showcase the increasing prevalence of NPS benzodiazepine and fentanyl drug combinations and 2) to demonstrate why comprehensive toxicology testing for benzodiazepines should be undertaken in medicolegal death investigations involving fentanyl.

Methods: Biological samples from forensic toxicology investigations were submitted for analysis from agencies across the United States. Following routine toxicology testing, sample extracts (n=6,499; Q3 2019 to Q2 2022) were re-analyzed using a comprehensive non-targeted acquisition strategy on a SCIEX TripleTOF[®] 5600+ quadrupole time-of-flight mass spectrometer (LC-QTOF-MS). Resulting datafiles were processed using a targeted library database containing more than 950 traditional drugs, NPS, metabolites, and other relevant analytes. In total, 27 NPS benzodiazepines and 26 traditional benzodiazepines were included in the scope.

Results: Beginning in mid-2019, a large increase in positivity for NPS benzodiazepines was observed. Since Q3 2019, 3,587 samples have tested positive for fentanyl. In that fentanyl-containing subset, etizolam (n=1,597) and flualprazolam (n=833) were most commonly detected alongside fentanyl. This was followed by clonazolam (n=262), flubromazolam (n=169), flubromazepam (n=128), and bromazolam (n=118). Over this same period of time, no significant changes in the positivity of fentanyl with diazepam or alprazolam were observed, demonstrating that benzo-dope is largely comprised of new benzodiazepines. From Q1 2021 to Q1 2022, the proportion of detections for NPS benzodiazepines with fentanyl increased for etizolam (79% to 88%), flualprazolam (64% to 88%), flubromazolam (36% to 46%), flubromazepam (0% to 87%), and bromazolam (33% to 75%); the only drug to decrease slightly was clonazolam (46% to 39%). When examining NPS benzodiazepines as a subclass, this group of drugs was found in 69% of fentanyl positive cases since mid-2019 (and 82% in Q1 2022 alone).

Conclusion/Discussion: Benzo-dope is an emergent and sustained drug supply phenomenon that has led to a large increase in combinations of opioids and benzodiazepines in forensic samples. While the data presented result from forensic toxicology cases, it should be noted that benzo-dope drug materials have contained combinations of NPS benzodiazepines and opioids (e.g., fentanyl, isotonitazene, *N*-pyrrolidino etonitazene) in individual dosage units – an important aspect for determining combined vs. concurrent use. Benzodiazepines are known to cause additive CNS depressant effects when ingested in combination with opioids. Given the risk of death when taken in combination with opioids, benzodiazepines (specifically new or emerging benzodiazepines) should be tested for in medicolegal death investigation cases, and toxicologists should consider their significance along with case history, autopsy findings, and drug material testing results, if available.

S28 - Hydromorphone and hydromorphone-3-glucuronide in blood: environmental impact of developed procedure

Cynthia Coulter,^a Margaux Garnier,^a **Christine Moore**,^{*a} Kelly E. Dunn,^b Ryan Vandrey,^b Claudia M. Campbell^b. a. 9-Delta Analytical LLC, Ontario, CA 91761.

b. Johns Hopkins University School of Medicine, Baltimore, MD 21224.

Background/Introduction: Hydromorphone (HYM) is a semi-synthetic narcotic analgesic opioid used for the relief of moderate to severe pain. It is metabolized in the liver, with >95% metabolized to hydromorphone glucuronide (H3G); in this procedure the glucuronide is analyzed with the parent drug without using hydrolysis, so as to minimize the inclusion of biological enzymes or harsh acids.

Objectives: One objective was to ensure a "green" procedure was used for determining HYM and H3G in blood. The environmental impact of the assay was assessed via two separate approaches: 1) Using the Complex green analytical procedure index (ComplexGAPI) which covers the entire analytical procedure, including sample collection, transport, preservation, and storage then compares the new method to an already published procedure; and 2), by using the Analytical Eco-Scale assessment (ESA) which calculates a numerical score by assigning penalty points (PP) in a method. PP are assigned to solvents, hazards, energy depletion, and waste generation.

Methods: To calibrators, controls, and specimens, 2% NH₄OH in deionized water was added; capped and vortexed. The samples were loaded onto solid-phase columns, washed with 2% NH₄OH solution then dried. HYM and H3G were eluted with methanol and evaporated under nitrogen at 40°C. The extracts were reconstituted in water, vortexed, transferred to autosampler vials, capped, and analyzed using LC-MS/MS in multiple reaction monitoring mode; two ion transitions were monitored for each drug.

Results: The method was validated and applied to blood specimens taken from adults with knee osteoarthritis in four experimental sessions. The first session administered 4mg of oral hydromorphone. Subsequent sessions consisted of 1 of 3 conditions, and order was randomized and counter balanced. Conditions were 1) placebo; 2) oral dronabinol (10mg); and 3) combined hydromorphone (4mg) and dronabinol (10mg). 11 participants underwent blood draws. The concentrations of HYM detected ranged from 1 - 4 ng/mL; The highest concentration of H3G detected was 55ng/mL in the specimen collected 1.5 hours after oral dosing; the corresponding HYM concentration was 2ng/mL.

ComplexGAPI software: The method was compared to a recently published procedure. Certain features of the comparative method were not included in pictogram generation (such as derivatization and additional elution solvents) since the drug requiring such treatment was not HYM or H3G and not relevant to the comparison. The developed procedure was pictorially "greener" than the recently reported method (to be shown in presentation/poster).

ESA: Penalty points for the method were assessed.

Reagents/Instruments	Penalty points
10% Ammonium hydroxide	
(Lowest available concentration)	4
Deionized water	0
Methanol	4
Formic acid	6
Energy depletion: LC-MS/MS	2
Occupational hazard (physical, health)	0
Waste (< 2 mL per sample)	3
Total penalty points	Σ19
Analytical Eco-Scale Total Score	81

The overall score for the developed procedure was 81. A green analysis is ideal if it has an Eco-Scale value of 100, excellent if >75, therefore the developed procedure is considered excellent.

Conclusion/Discussion: A green, rapid, sensitive LC-MS/MS method was developed and validated for the quantification of HYM and H3G in blood. The method minimized environmental impact by using small extraction columns, non-halogenated solvents, low wash volumes, and buffer/salt free mobile phases. Scientists should endeavor to develop procedures which are efficient and environmentally friendly.

S29 - Challenges associated with cross-validating analytical methods across multi-generational LC/MS/MS platforms in the forensic toxicology laboratory

Britni Skillman, PhD*; Crystal Arndt, MSFS, D-ABFT-FT; Teresa R. Gray, PhD, F-ABFT. Harris County Institute of Forensic Sciences, Houston, TX 77054.

Background/Introduction: As the scope of testing in forensic toxicology laboratories widens and technological advances are made to analytical techniques, instrumentation upgrades are not uncommon and older versions of equipment may be phased out of service. As such, when a new instrument is purchased, the laboratory must take measures to verify its performance. Current validated assays can be transferred to alleviate instrument burden and serve as backup in the event of equipment failure or end of service for older models. A verification must be performed to demonstrate equivalency between instruments, in which case an abbreviated cross-validation may be performed. However, improvements to instrument performance between different generations of platforms may introduce challenges to the abbreviated validation process, such as improved sensitivity, thereby increasing response, and differing efficiencies of fragmentation. These factors can affect calibration model, limits of detection and quantitation, and ion/transition ratios. This research describes analytical challenges associated with the transfer of various analytical methods between two liquid chromatography tandem mass spectrometry (LC/MS/MS) instruments of different generations and the resolution of conflicts encountered during the cross-validation of these methods.

Objectives: To address challenges that may be encountered during abbreviated cross-validation versus full redevelopment and validation of analytical methods to newer generations of instrumentation

Methods: Several analytical methods were evaluated on an existing Agilent 6460 Triple Quadrupole LC/MS/MS system and a new Agilent 6470 Triple Quadrupole LC/MS/MS. Individual assays included acetaminophen, opiates, opioids, promethazine, diphenhydramine-trazodone-buspirone, psychotropic drugs, and stimulants. At minimum, methods were evaluated for bias, precision, carryover, limit of detection, limit of quantitation, and calibration model equivalency over at least three days. Previously validated liquid-liquid and solid phase extraction techniques were used for blood, liver, stomach contents, vitreous humor, brain, and urine, depending on the assay. Initial quantitative performance was monitored to evaluate the need for method modifications. When method changes impacted parameters affecting quantitation (particularly calibration model or ion selection) a full method validation followed to obtain a minimum of five days of quantitative data.

Results: Of seven methods assessed, the promethazine assay was evaluated with an abbreviated cross-validation following only a minor dwell time modification. As a result of increased sensitivity, opiates, diphenhydramine-trazodone-buspirone, and stimulants assays were fully validated across five days due to calibration model changes for some analytes, Acetaminophen and opioids methods were redeveloped to improve poor performance over the calibration range, resulting in two full validations which also included interference, ion suppression, and processed sample stability studies. The psychotropic drug assay was postponed due to inability to achieve method equivalency despite many modifications, primarily due to concentration-dependent ion ratio behavior as result of instrument sensitivity differences. The most common method modifications were to injection volumes, dwell times, collision energies, cell accelerator voltages, and in some instances a change to precursor or product ion selection and corresponding fragmentor voltages. Performance of revalidated methods was deemed equivalent or improved. For fully redeveloped methods, efficiency improvements were made.

Conclusion/Discussion: Parameters that can impact successful replication of a method between two generations of instruments can include fragmentation parameters, data acquisition and detection settings, and factors impacting precursor ion response. As instrumentation advances and new platforms are introduced, the adaptability of existing methods is an important factor, especially considering the ever-changing scope of testing requirements. All of these challenges should be considered when deciding to perform verification of methods versus full validation in context with the needs of the laboratory.

S30 - Getting high (throughput) on psilocin

Glenna J. Brown*, Madeleine E. Wood, Joshua Z. Seither, Jordan T. Brown, Jessica L. Knittel, Erin L. Karschner, and Jeffrey P. Walterscheid. Division of Forensic Toxicology, Armed Forces Medical Examiner System, 115 Purple Heart Dr., Dover AFB, DE 19902.

Background/Introduction: Recently, requests for the screening and confirmation of psychedelic mushroom use in forensic toxicological investigations have increased at the Armed Forces Medical Examiner System Division of Forensic Toxicology Laboratory. Psilocybin is the component of interest in psychedelic mushrooms. Although inactive, psilocybin is rapidly dephosphorylated to psilocin after ingestion and conjugated with glucuronide. Psilocin is a 5-HT_{2A} receptor agonist that produces hallucinogenic effects and emotional mood swings, along with impaired perception, making it an analyte of interest in forensic toxicological investigations. Analytical challenges must be overcome to appropriately identify and confirm the presence of psilocin in human specimens. The most notable challenges include analyte stability and the fact that human specimens can contain significant basal concentrations of bufotenine, which is an endogenous isomer that can complicate detection of psilocin if the two compounds are not properly resolved by chromatography.

Objectives: This presentation will describe the development and validation of a high-throughput screening method to identify psilocin in human urine and the development and validation of a qualitative confirmation method for psilocin in human blood and urine.

Methods: Prior to extraction, Escherichia coli β -glucuronidase was used to hydrolyze the psilocin-*O*-glucuronide bond to enrich unbound psilocin in urine specimens. A solid phase extraction (SPE) protocol using a Hamilton STAR automated liquid handling system equipped with DPX Technologies XTR tips (10 mg SCX, 60 µm) was developed and validated. The automated SPE procedure included steps to bind, wash, and release the purified target compound for analysis. A liquid chromatography tandem mass spectrometry (LC-MS/MS) confirmation method and offline SPE method were also developed and validated to confirm the presence of psilocin in blood and urine. Validation of both methods followed ANSI/ASB Standard 036. The validated methods were used to screen and confirm the presence of psilocin in authentic specimens from forensic toxicological investigations. Total specimen volume for screening and confirmation testing was reduced from 8 mL using previous methods to 1 mL. Both screening and confirmation LC-MS/MS methods were capable of resolving psilocin from bufotenine at a limit of detection (LOD) of 5 ng/mL.

Results: LODs in 3 different matrix sources over 3 days met all acceptance criteria and internal standard normalized matrix effects were ≤0.7% for both screening and confirmation methods. No exogenous or endogenous interferences were observed in either method. Since 2019, psilocin was confirmed in 58 cases submitted to the laboratory for forensic toxicological investigations where psychedelic mushroom use was suspected. Automation of the screening method increases speed and accuracy of testing by decreasing total extraction time and providing increased quality assurance of results. Blood psilocin cases benefitted from frozen storage, which provided better analyte stability, resulting in extended analysis time frames. Furthermore, the addition of ascorbic acid protected psilocin from further oxidation and increased stability during sample preparation.

Conclusion/Discussion: Screening automation allows for faster casework throughput and enhanced quality assurance, improving turnaround time. Compared to previous in-house methods, specimen volume was substantially decreased for both blood and urine, providing advantages for testing when sample volume is limited. This technique is well-suited for evaluating large numbers of specimens from those who are employed in safety-sensitive workforce positions. The increasing number of psilocin confirmations in investigative casework demonstrates the on-going need to have adequate screening and confirmation methods for psilocin when psychedelic mushroom usage is suspected.

S31 - Development of a supported liquid extraction (SLE) high resolution mass spectrometry-based drug screening protocol for comprehensive toxicological analysis of whole blood

Jessica Ayala*, M.S., D-ABFT-FT and Sarah Kerrigan, Ph.D.

Department of Forensic Science, Sam Houston State University, Huntsville, TX.

Introduction: Comprehensive drug screening protocols in forensic toxicology laboratories have become an important issue due to the persistence of new psychoactive substances (NPS). Guidelines for the scope and sensitivity for testing in drug-impaired driving (DUID) investigations were first published in 2007 and updated recently in 2021¹. As a result, laboratories are encouraged to develop broad screening techniques for common drugs of abuse, therapeutics, and NPS. This is difficult to achieve with traditional immunoassay (IA)-based methods alone. Therefore, mass spectrometry-based approaches, including high resolution mass spectrometry (HRMS) have gained widespread attention. However, this approach is complex, and comprehensive extractions in whole blood are challenging due to the differences in physicochemical properties (*e.g.,* polarity and acid-base characteristics).

Objectives: The purpose of this study was to develop a comprehensive screen in whole blood using liquid chromatography quadrupole time-of-flight mass spectrometry (LC-QTOF-MS) for application to DUID investigations that meets or exceeds recommendations for toxicological analysis.²

Methods: Method development began with instrumental analysis using reference standards to optimize sample injection, chromatographic resolution, source ionization, and data acquisition. A representative group of drugs including those with challenging cutoff concentrations, isomers, and difficult drug classes (*i.e.*, cannabinoids) were selected for preliminary investigation. Negative and positive electrospray ionization (ESI) were evaluated in addition to fast polarity switching (FPS). Three modes of data acquisition (*i.e.*, All lons, Auto MS/MS, and TOF) were assessed to identify the optimal technique for drug detection by comparing average values for mass accuracy (MA), target score, and signal-to-noise ratio (SNR).

A variety of sample preparation techniques were evaluated including protein precipitation (PPT), phospholipid removal (PR), dispersive solid phase extraction (dSPE), and SLE. In addition, SLE was evaluated alone, and in combination with PPT and PR. Traditional and synthetic sorbents for SLE were investigated, in addition to extraction conditions/solvent, diluent, sample size, extraction consumables (*i.e.*, glass, plastic), and centrifugation.

Results: Optimum results were achieved with dual injection analysis using both positive and negative ESI versus FPS. No significant differences in data acquisition were determined in negative ESI, although significant differences in MA and SNR were observed using positive ESI. Data was statistically evaluated, and All Ions was selected over Auto MS/MS and TOF modes. Less dispersion of results and increased SNR were achieved using All Ions.

11-Nor-9-carboxy-delta-9-tetrahydrocannabinol (THC-COOH) is one of the most challenging compounds to isolate in a comprehensive extract. SLE was the only technique capable of recovering THC-COOH in combination with all other analytes at the recommended cutoff concentrations. The addition of PPT and PR to SLE analysis did not improve overall performance. An acidic sample diluent was chosen to improve analyte recovery for cannabinoids. The optimum elution contained a mixture of hexane, ethyl acetate, and isopropanol (70:23:07) using traditional SLE columns.

Conclusion: Laboratories routinely have separate screening assays for cannabinoids because "comprehensive" extraction protocols often have poor recovery for critical compounds like THC-COOH. The optimized procedure could identify >200 compounds in blood at the recommended cutoffs using dual injection LC-QTOF-MS and All Ions acquisition in positive and negative ESI. It met published recommendations for the scope and sensitivity of testing in DUID investigations and possesses additional benefits for analyte identification and NPS discovery (*i.e.,* retrospective data analysis). Further studies were conducted separately to validate this procedure in accordance with published toxicological standards³.

3 ANSI/ASB Standard 036. Standard Practices for Method Validation in Forensic Toxicology (2019).

¹ D'Orazio, Amanda L., et al. "Recommendations for Toxicological Investigation of Drug-Impaired Driving and Motor Vehicle Fatalities—2021 Update." *Journal of Analytical Toxicology* 45.6 (2021): 529-536.

² ANSI/ASB Standard 120. Standard for the Analytical Scope and Sensitivity of Forensic Toxicological Testing of Blood in Impaired Driving Investigations (2021).

S32 - Using the EZSTATSG3 validation tool to make your life easier and your validations traceable

Jocelyn V. Abonamah^{1*}, Szabolcs Sofalvi², and Harold E. Schueler^{3. 1}Cleveland Clinic Foundation, Cleveland, OH, ²Cuyahoga County Medical Examiner's Office, Cleveland, OH, ³Ohio Northern University, Ada, OH.

Background/Introduction: Processing method validation data can be complicated and time-consuming, which may deter forensic laboratories from completing comprehensive validations. Obtaining the data to meet the ASB Standard 036 guide-lines is not difficult, but processing and assessing the data may be a barrier for some laboratories. To try to ease this burden, a Microsoft (MS) Excel tool was developed. EZSTATSG1 was designed for methods requiring seven calibration points and included six weighted linear calibration models with standardized residuals, bias and precision data, dilution integrity, and ion suppression. EZSTATSG2 summarizes all the validation parameters of a method and incorporates flexible five-, six-, or seven-point calibration curves. G2 also includes six weighted quadratic calibration models, standardized residuals by use of frequency plots, and processed sample stability. When EZSTATSG3 is released, it will feature automatic data importation from several vendors, an option for Method of Standard Addition, and improved tools for model order and weight selections.

Objectives: The goal of this presentation is to emphasize why complete validations following the ASB Standard 036 guidelines are important for all forensic toxicology laboratories, familiarize attendees with EZSTATS by demonstrating how the template works in real time and how to interpret output data, and share how EZSTATS can help laboratories feel confident when processing validation data.

Methods: EZSTATSG2 includes six weighted linear and quadratic calibration models, flexible number of calibration levels, LODs estimated from linear calibration curves and measured by reference material, bias and precision data for the LLOQ, QCs and dilution integrity using ANOVA, carryover, recovery, ion suppression, standardized residuals, and processed sample stability. The quadratic models feature 95% confidence intervals for checking the significance of the second order term. The addition of Visual Basic for Applications (VBA) enables macros that facilitate data entry and transfer, lock the cells for security purposes, and auto-save both Excel and PDF files. EZSTATS automatically performs all statistical calculations required by the ASB Standard 036 for each method validation parameter. The version in development includes the addition of data importation from the four major vendors ThermoFisher, Agilent, Waters, and Sciex.

Results: A major improvement to EZSTATSG1 was the implementation of a flexible number of calibration levels rather than the fixed seven. The second significant improvement was the inclusion of weighted quadratic calibration models in addition to the linear weighted models present in the previous version. A histogram of residuals overlaid with the normal probability density function helps assess the normality of the residuals. A tab was also created to evaluate processed sample stability. The implementation of VBA UserForms in EZSTATSG2 prevents accidental alteration of existing formulas, and integration of the data import feature in EZSTATSG3 significantly decreases the time spent entering calibration data.

Conclusion/Discussion: The ANSI/ASB Standard 036 states "the simplest calibration model that best fits the concentration-response relationship should be used"; however, the simplest calibration model is not always the best fit. The best curve fit model representing the trending data should be used for a newly developed method. EZSTATSG2 is the first comprehensive MS Excel tool that can help forensic toxicology laboratories choose the best curve fit model. The current template provides an independent verification of both linear and quadratic weighted calibration models regardless of instrument manufacturers' software platforms, and EZSTATSG3 will integrate data importation for significant time savings. The current version of EZSTATS is available at <u>www.EZSTATS4validation.com</u>.

S33 - Use of common fragments and neutral losses to streamline screening workflows for the detection of novel psychoactive substances in forensic toxicology casework

Joshua S DeBord, PhD^{*1}, Alex J Krotulski, PhD¹, Barry K Logan, PhD^{1. 1}The Center for Forensic Science Research and Education, Fredric Rieders Family Foundation, 2300 Stratford Ave, Willow Grove, PA.

Background/Introduction: The landscape of potentially harmful, novel psychoactive substances (NPS) is ever changing. For a forensic toxicology lab, keeping validated analytical methods current is not only expensive and intensive on the workforce, but it can needlessly strain resources when a particular drug falls out of use. An alternate approach is to keep screening methods flexible for analytes within the classes of drugs known to frequently change. It may be more efficient to simply identify the class of a low-frequency NPS rather than spend the money, time, and effort to validate targeted, or drug-specific, screening methods. High-resolution tandem mass spectrometry enables analysts to acquire an unknown drug's precursor accurate mass, its fragment masses, and the resulting neutral mass differences, allowing for the prediction of a particular subclass for the unknown; thus avoiding full identification and structural elucidation of the unknown drug in the screen.

Objectives: The presentation's objective is to illustrate how a workflow designed with a moderate number of mass filters for common fragments (m/z) and neutral losses (NL) can enable presumptive screening for fentanyl analogs, nitazene analogs, and synthetic cannabinoids, and secondarily to discuss limitations of such an approach.

Methods: Standard reference materials were purchased from Cayman Chemical on a routine basis for addition to an inhouse high resolution mass spectral library database. Standards were prepared at 1 ng/µL in mobile phase and analyzed using a SCIEX TripleTOF® 5600+ quadrupole time-of-flight mass spectrometer coupled to a Shimadzu Nexera high-performance liquid chromatograph (LC-QTOF-MS). The LC gradient was 5% B at start, 5% B at 1 min, 95% B at 10 min, 95% B at 13 min, 95% B at 13.1 min, and stopped at 15 min, using a mobile phase A of 10mM ammonium formate (pH=3) and a mobile phase B of 0.1% formic acid in 50:50 methanol:acetonitrile. Information-dependent acquisition mode was used to acquire the mass spectral data. Resulting MS/MS fragment spectra were consolidated in LibraryView[™] (SCIEX) and recorded in an Excel spreadsheet. The library was queried to identify the most frequently observed common fragment ions for fentanyl analogs, nitazene analogs, and synthetic cannabinoids. The neutral losses associated with the fragment ions were calculated by the mass difference from the precursor masses.

Results: The presentation describes three separate workflows that each use N \leq 10 common fragments and neutral losses with good efficiency for identifying unknown NPS. Efficiency was evaluated as having N \geq 2 targets met for each drug and a coverage of \geq 80% of analytes within the developed NPS Discovery (CFSRE) library. Workflow summaries for fentanyl analogs, nitazene analogs, and synthetic cannabinoids along with structural examples are given. For example, 100% coverage of N=13 library nitazene analogs was achieved by monitoring the common fragments (72.09, 100.11, and 107.05 m/z) and the neutral losses (262.15, 297.12, and 325.15 NL). Mass tolerances of \pm 10 mDa are recommended for this workflow to avoid false negatives.

Conclusion/Discussion: This research will provide a means for forensic toxicologists to presumptively identify the presence of out-of-scope NPS in casework. The proposed workflows will enhance the ability of toxicology laboratories to identify and predict the class of a NPS that is outside of their analytical scope. Having this information, the lab can better determine if there is a need for confirmation and, if so, how best to perform the secondary analysis. With ever-changing NPS trends, including a workflow step for screening based upon common ion fragments and neutral losses can decrease the chance that a laboratory misses the identification of an unknown drug.

S34 - Analysis of commercial Δ⁸-THC products for microbiologics and phytocannabinoid concentrations

Kimberly N. Karin^{1*}, Michelle R. Peace², Aron H. Lichtman¹, Justin L. Poklis^{1,1}Department of Pharmacology and Toxicology, ²Department of Forensic Science, Virginia Commonwealth University, Richmond, VA.

Background/Introduction: As a result of the ambiguity around the legality of hemp-derived tetrahydrocannabinol (THC) isomers and derivatives, Δ^{8} -THC products recently flooded the market. During the growing and processing phases, microbes, metals, and pesticides may contaminate these hemp-derived products leading to a significant health risk to consumers. As evidence, the National Poison Control reported 2,362 cases with Δ^{8} -THC related products between January 2021 and February 2022, 70% of which needed to be evaluated by healthcare professionals. This shows concern for consumers who may experience adverse health effects due to these products or their contaminants.

Objectives: Commercially available Δ^{8} -THC gummies, e-liquids, and plant material were assessed for the presence of microbiologics including aerobic bacteria, *Escherichia coli* (*E. coli*), yeast, and mold using 3M PetrifilmTM Count Plates. Additionally, quantitation of phytocannabinoids was performed in the Δ^{8} -THC products.

Methods: In order to test for the presence of microbiologics, 100 mg of product was extracted in 5 mL of distilled water and agitated in a beadruptor for 1 min. Samples and a negative control of distilled water were plated (1 mL each) on 3M Petrifilm *E. Coli*, Aerobic Count, and Yeast and Mold plates. A 1:10 dilution in distilled water was also plated for plant material. Colonies were manually counted after a 24 hr incubation period at 32°C.

For the quantitation of phytocannabinoids, 200 mg of product was extracted in 5 mL of 1:1 acetonitrile: water for gummy material or 5 mL of acetonitrile for e-liquids and plant material. The extract was then agitated in a beadruptor for 1 min. E-liquid and gummy extracts were diluted 1:1000 in methanol, while plant material extracts were diluted 1:10,000 in methanol. The diluted samples were analyzed using a Sciex ExionLC 2.0 liquid chromatograph attached to a Sciex 6500 QTRAP system with an IonDrive Turbo V source for TurbolonSpray. Analytes were separated on a Zorbax Eclipse XDB-C18 column with 10:90 water: methanol with 0.1 mM ammonium formate delivered at a flow rate of 1 mL/min. The following transitions were monitored in multiple reaction monitoring with: cannabichromene/ Δ^9 -THC/ Δ^8 -THC/cannabidiol 315>123 & 315>193; cannabinol 311>223 & 311> 241; cannabigerol 317>123 & 317>193; cannabidivarin/tetrahydrocannabivarin 287>165 & 287>231; CBDAA/THCAA 359>219 & 359> 341; and CBD-d₃/THC-d₃ 318>123 & 318>193.

Results: While none of the gummies (n=3) nor the e-liquid (n=1) tested positive for a microbiologic above any regulatory limit in the United States, two of the four plant products tested positive. One plant product, Grease Monkey, displayed yeast and mold growth on the plate with 547 counts (287,000 cfu/g). Aerobic bacteria growth was observed for the Moon MCU plant product with 240 counts (120,000 cfu/g). The negative controls samples did not show growth on any of the plates.

 Δ^8 -THC concentrations in the three different gummies ranged from 3.5 to 6.1 mg/g, with all the gummies containing less than the reported dose (-37 to -65% difference). While the gummies contained less than 0.3% Δ^9 -THC, one of the products contained double the reported value. Of the four plant materials assessed, all products contained greater than 0.3% Δ^9 -THC, ranging from 0.38-1.0%. The Δ^8 -THC concentrations of the plant materials ranged from 0.97-1.2%. Only one plant product had labeled Δ^8 -THC concentration, which was -78% different from the quantitated concentration. While the e-liquid claimed to contain 96% Δ^8 -THC, the concentration was determined to be 21% with 4% Δ^9 -THC.

Conclusion/Discussion: With the lack of regulation, the presence of harmful contaminants, including microbes, pose a risk to the safety of consumers. Additionally, unregulated products continue to contain doses of phytocannabinoids inconsistent with labeling.

Funding: Funded in part by National Institute of Health (P30DA033934 and T32DA007027).

S35 - The aerosolization efficiency of cocaine in a eutectic mixture with nicotine in electronic cigarettes

Laerissa Reveil*¹, **M.S.**, Justin Poklis², B.S., Matthew S. Halquist³, Ph.D., Michelle R. Peace¹, Ph.D. ¹Department of Forensic Science, Virginia Commonwealth University, Richmond, VA, ²Department of Pharmacology & Toxicology, Virginia Commonwealth University, Richmond, VA, ³Department of Pharmaceutics, Virginia Commonwealth University, Richmond, VA.

Background/Introduction: E-liquid formulations usually contain humectants, such as propylene glycol (PG) and vegetable glycerin (VG), a pharmacologically active ingredient, such as nicotine, and flavoring agents. With the rise of electronic cigarette (e-cigarette) use and the technological advancements of these devices, users can adulterate e-liquids with drugs other nicotine (DOTNs), such as ethanol, herbal products, or illicit substances. The interaction between nicotine and the diluent forms a eutectic mixture, which reduces the melting temperature to a point lower than the temperature of the individual compounds and promotes aerosolization. Previous studies have demonstrated mixtures of equal parts nicotine and methadone resulted in increases in the drug dose in the aerosol, as compared to the single drug e-liquids.

Objectives: The purpose of this study was to evaluate the effect of nicotine on the aerosolization efficiency and recovery of cocaine using an automated vaping machine and an aerosol capture method adopted from the industry standard method established by the Cooperation Centre for the Scientific Research Relative to Tobacco (CORESTA) E-cigarette Task Force and a validated gas chromatography-mass spectrometry (GC-MS) method.

Methods: Individual e-liquids were prepared with 12 mg/mL nicotine and cocaine hydrochloride. Aerosol capture was performed on four e-liquids, 1:1 PG:VG (drug-free), 100% cocaine hydrochloride in 1:1 PG:VG, 100% nicotine in 1:1 PG:VG, and 1:1 cocaine hydrochloride:nicotine in 1:1 PG:VG, using parameters adopted from the CORESTA method. Briefly, the puff profile determined for the aerosol capture method was an inhale duration of 3 seconds, an exhale duration of 10 seconds, and a puff volume of 60 mL for a total of 15 puffs. Cocaine quantitation was validated on a Gas Chromatograph-Mass Spectrometer (GC-MS) using guidelines outlined in the ANSI/ASB Standard Practices for Method Validation in Forensic Toxicology over five days. The linear range for the calibration curve was 50 - 5000 ng/mL. Linearity was evaluated using quality controls at 150, 1500, and 4000 ng/mL, which were assessed for bias, precision, and carryover. Bias and precision were -4% (\pm 4 %CV) for 150 ng/mL, -3% (\pm 4 %CV) for 1500 ng/mL, and 6% (\pm 2 %CV) for 4000 ng/mL, and no carryover was observed. Aerosol samples were analyzed for recovery and drug dose using the validated GC-MS method. Samples were also screened for the pyrolytic products of nicotine and cocaine: **β**-nicotyrine for nicotine and benzoic acid and anhydroecgonine methyl ester (AEME) for cocaine.

Results: In the single drug e-liquids prepared at 12 mg/mL, 0.22 g (\pm 8 %CV) of nicotine and 0.15 g (\pm 10 %CV) of cocaine was aerosolized (N=3). In the mixed drug e-liquid, the amount of drug aerosolized increased to 0.38 g (\pm 6 %CV). Nicotine and cocaine had recoveries of 77% (\pm 11 %CV) and 66% (\pm 11 %CV) in the single drug e-liquids. The recoveries of both nicotine and cocaine increased in the mixed drug e-liquid to 192% (\pm 25 %CV) and 108% (\pm 6 %CV), respectively. Pyrolytic products were not observed as a result of aerosolization. They were identified in the e-liquids and aerosol samples.

Conclusion/Discussion: The eutectic mixture can result in an increase in aerosolized drug doses. This trend is consistent with the resulting increased aerosolization efficiency of methadone in a eutectic mixture with nicotine. Users combining drugs in an e-liquid may experience adverse reactions. The formation of pyrolytic products did not impact the aerosolization efficiency of the parent drugs; however, their presence may cause unexpected effects.

S36 - Pharmacology and toxicology of *N*-pyrrolidino etonitazene – a new nitazene synthetic opioid increasingly observed in forensic cases

Sara E. Walton^{1*}, Marthe M. Vandeputte², Alex J. Krotulski¹, Donna Walther³, Grant C. Glatfelter³, Donna M. Papsun⁴, Barry K. Logan^{1,4}, Michael H. Baumann³, Christophe P. Stove². ¹Center for Forensic Science Research and Education at the Fredric Rieders Family Foundation, Willow Grove, PA ²Laboratory of Toxicology at Ghent University, Belgium ³Designer Drug Research Unity, National Institute on Drug Abuse, Baltimore, MD ⁴NMS Labs, Horsham, PA.

Background/Introduction: The rise of non-fentanyl synthetic opioids in forensic casework has increased in the recent years, with the fastest growing subclass being the 2-benzylbenzimidazoles (or the "nitazenes"). Isotonitazene was the first drug in this subclass to be identified in forensic samples in 2019; however, since the emergence of isotonitazene, eight additional nitazenes have been reported with little knowledge of their pharmacological activity and toxicity. The most recent proliferation of *N*-pyrrolidino etonitazene (etonitazepyne), an opioid much more potent than fentanyl, has been an increasing public health concern demonstrated by the need for continuous detection and pharmacological evaluation of this opioid subclass.

Objectives: The objective of this study was to pair *in vitro* and *in vivo* methodologies to characterize the new synthetic opioid, *N*-pyrrolidino etonitazene. This was achieved by completing radioligand binding assays and activation potential assays, pharmacodynamic studies (i.e., catalepsy, body temperature), as well as developing a validated method to quantify by standard addition the concentrations of *N*-pyrrolidino etonitazene in authentic biological samples using liquid chromatography tandem quadrupole mass spectrometry (LC-QQQ-MS).

Methods: For the *in vitro* and *in vivo* analyses, radioligand binding assays in rat brain tissue using three different radioligands ([³H]DAMGO, [³H]DADLE, [³H]U69593) and NanoBiT^{*} MOR- β -arrestin-2 cell-based recruitment assays to determine MOR activation potential were performed. Pharmacodynamic studies were performed using Male Sprague-Dawley rats, and procedures for catalepsy, body temperature, and hot plate latency were carried out after administration of differing doses of *N*-pyrrolidino etonitazene (0.0003-0.010 mg/kg). For toxicological analysis of *N*-pyrrolidino etonitazene, initial drug screening was completed using a Sciex TripleTOF 5600+ liquid chromatograph time-of-flight mass spectrometer (LC-QTOF-MS). For confirmation, a method employing standard addition was developed and validated. A basic liquid-liquid extraction was used consisting of borax buffer (1 mL, 10 mM, pH 10.4) and extraction solvent (3 mL, 70:30 *N*-butyl chloride/ ethyl acetate). Quantitative analysis was performed using a Waters Xevo TQ-S Micro LC-QQQ-MS. Chromatographic separation was achieved using an Agilent InfinityLab Poroshell C-18 (2.7 µm, 3.0 x 100 mm) column using gradient elution. The flow rate was 0.4 mL/min (mobile phase compositions: 0.1% formic acid in water and 0.1% formic acid in methanol). The injection volume was 5 µL and the column temperature was 30°C. For standard addition the peak area ratios were plotted against the up-spike values to determine the concentration of *N*-pyrrolidino etonitazene. This workflow has been applied to numerous authentic samples since its implementation.

Results: *N*-Pyrrolidino etonitazene was determined to have a Ki of 4.09 ± 0.63 nM at the MOR, showing greater affinity to MOR than fentanyl. The *in vitro* activation potential of *N*-pyrrolidino etonitazene was similar to etonitazene and the potency (EC₅₀: 0.348 nM) was much greater than both morphine and fentanyl; 800x and 40x, respectively. The analgesic effect of *N*-pyrrolidino etonitazene was ~10x greater than that of fentanyl, depicting significant effects on all pharmacodynamic studies completed. *N*-Pyrrolidino etonitazene was confirmed in 21 postmortem cases collected from January-October 2021. This opioid was found in combination with other opioids and stimulants, but also with a wide variety of novel benzodiazepines (suggesting potential benzo-dope drug materials). The range of concentration for *N*-pyrrolidino etonitazene in blood was 0.3-25 ng/mL (mean: 2.5±2.0 ng/mL, n=13), which is comparable with the low concentrations observed with other 2-benzylbenzimidazole analogues.

Conclusion/Discussion: Using this paired approach of *in vivo* and *in vitro* characterization of *N*-pyrrolidino etonitazene, it was determined that this new synthetic opioid is an extremely potent MOR agonist with high MOR activation potential and produces significant analgesic effects. *N*-Pyrrolidino etonitazene was discovered in 21 postmortem cases and quantitated at low concentrations, showing the need for sensitive methodology. The continued proliferation of *N*-pyrrolidino etonitazene depicts the risks to public health and safety.

S37 - Developing a unified method for the analysis of five ethanol metabolites: EtG, EtS, GTOL, 5-HTOL, and 5-HIAA using UPLC-MS/MS

Bailey K. Jones, B.S.^{*1}, Alaina K. Holt, B.S.¹, Nicholas R. Rhodes, Ph.D.², Sue Brown, Ph.D.², Justin L. Poklis, B.S.³, Michelle R. Peace, Ph.D¹.

¹Department of Forensic Science, Virginia Commonwealth University, Richmond, VA, 23284;

²Paradigm Labs, LLC. St. Simons Island, GA, 31522;

³Department of Pharmacology and Toxicology, Virginia Commonwealth University, Richmond, VA 23284.

Background/Introduction: Given that approximately 1.5 million people were arrested in 2019 for alcohol-related crimes, ethanol analyses comprise the largest category of quantitative assessments and impairment interpretations in the forensic toxicology community. Biomarkers have been used as tools to detect ethanol use and aid in the assessment of recent alcohol use or past at-risk drinking behavior. Existing literature describes fit-for-purpose methodology for single or paired ethanol metabolites. Standard clinical cut-off concentrations for ethyl glucuronide (EtG) and ethyl sulfate (EtS) are 500 ng/ mL and 100 ng/mL, respectively. 5-hydroxytryptophol (5-HTOL), 5-hydroxytryptophol glucuronide (GTOL), and 5-hydroxy-indole-3-aceteic acid (5-HIAA) are represented in the form of two ratios (GTOL/5-HIAA and 5-HTOL/5-HIAA). The current clinical cut-off concentrations for ethyl are 15 nmol/µmol and 15 pmol/nmol, respectively.

Objectives: The purpose of this study was to develop, and validate, a single method for the quantitation of EtG, EtS, GTOL, 5-HTOL, and 5-HIAA in urine using UPLC-MS/MS, following the AAFS Standards Board (ASB) Standard 036 Practices for Method Validation in Forensic Toxicology to correlate the major direct biomarkers indicating alcohol exposure.

Methods: A single solid phase extraction (SPE) method was performed using United Chemical Technologies Clean Screen FAST EtG SPE columns (3mL, 200mg sorbent). In a test tube, 500 μ L of each urine sample, 50 μ L of a 1 μ g/mL working internal standard solution containing EtG-d5, EtS-d5, HIAA-d6, and 500 μ L of 0.1% formic acid in water were combined and extracted for analysis on a Shimadzu LC-MS/MS-8050. Chromatographic separation was performed on an Agilent InfinityLab Poroshell HPH-C18 column (4.6mm x 100mm x 2.7 μ m) at 55°C with an injection volume of 2 μ L. The mobile phase was 0.1% formic acid in water (A) and acetonitrile (B). A binary gradient was used with a flow rate of 0.5 mL/min, starting conditions of 5% B, and the following programming: 0.01-3.50 min: 35% B; 3.51-5.00 min: 35% B; 5.01-6.00 min: 5% B; 6.01-7.00 min: 5% B. The working range of the validated method is 100 – 5000 ng/mL for EtG, EtS, and GTOL, respectively, 10 – 2000 ng/mL for 5-HTOL, and 20 – 5000 ng/mL for 5-HIAA. A six to eight-point calibration curve was used to build the calibration model for each analyte. Clinical samples from Paradigm Labs, LLC, were analyzed. EtG and EtS were evaluated above and below the clinical cut-off concentrations for the 5-HTOL/5-HIAA and GTOL/5-HIAA ratio.

Results: The five analytes were chromatographically resolved. The limit of detection for EtG, EtS, GTOL, 5-HTOL, and 5-HIAA was determined to be 235, 29, 90, 4, and 13 ng/mL, respectively, and the lower limit of quantitation for EtG, EtS, GTOL, 5-HTOL, and 5-HIAA was determined to be 711, 87, 273, 11, and 38 ng/mL, respectively. For all calibrators and controls, the bias, within-run CV, and between-run CV were within \pm 20%. The ranges of EtG and EtS concentrations observed in the clinical samples were 0-191,998 and 0-62,022 ng/mL, respectively. The ranges of the GTOL/5-HIAA ratio and 5-HTOL/5-HIAA ratio observed were 0-17,051 nmol/µmol and 0-1702 pmol/nmol, respectively. No statistically significant differences were observed between the population means for EtG concentrations (p(1.49) = 0.14) and EtS concentrations (p(2.20) = 0.03) below and above the clinical cutoff for 5-HTOL/5-HIAA. Statistically significant differences between the population means for EtG concentrations (p(2.76) = 0.009) were observed below and above the clinical cutoff for GTOL/5-HIAA.

Conclusion/Discussion: A unified method for the quantitation of EtG, EtS, GTOL, 5-HTOL, and 5-HIAA was developed, and validated, using UPLC-MS/MS. The validated method successfully quantitated ethanol metabolites in clinical urine specimens of subjects who orally consumed ethanol.

S38 - Opioid-like adverse effects of tianeptine in male rats and mice

Tyson R. Baird^{*1,2}, Justin L. Poklis^{2,3}, Michelle R. Peace², S. Stevens Negus³. ¹Integrative Life Sciences Doctoral Program and Departments of ²Forensic Science and ³Pharmacology and Toxicology, Virginia Commonwealth University, Richmond, Virginia.

Background/Introduction: Tianeptine is a clinically approved antidepressant medication in many countries that has only recently been discovered to act as a mu-opioid receptor agonist. Since the discovery of its opioidergic activity, reports of abuse in human populations have increased. Due to the lack of federal regulatory control in the United States, it has appeared in retail stores and earned the moniker "gas station heroin." Although reports of tianeptine abuse have been increasing, the scope of its use and the associated risks are not fully understood.

Objectives: This study was a risk assessment of the drug tianeptine, focused on its ability to induce adverse effects typical of mu-opioid agonists, including abuse potential, respiratory depression, locomotor impairment, and inhibition of gastro-intestinal transit.

Methods: Abuse potential of tianeptine was assessed in male Sprague-Dawley rats using an intracranial self-stimulation (ICSS) procedure which involves electrical activation of brain reward areas. The effects of acute intraperitoneal (IP) tianeptine on responding for electrical brain stimulation was determined at doses of 1.0 to 32 mg/kg over a 100-minute period. Tolerance and dependence were assessed by evaluating the effects of repeated daily IP tianeptine over a 1-week period on ICSS. Respiratory depression was assessed by monitoring changes in tidal volume and breath rate following the administration of high-dose subcutaneous tianeptine (100 mg/kg) in male Swiss-Webster mice using whole-body plethysmography. Gastrointestinal motility (100 mg/kg) and horizontal locomotor behavior (10-100 mg/kg) were assessed in male ICR mice.

Results: Tianeptine (10 mg/kg, IP) produced transient and delayed ICSS facilitation in rats, with a short duration of action, but its primary acute effect was naltrexone-preventable depression of ICSS rates (32 mg/kg, IP). Repeated administration of tianeptine over a 1-week period produced modest tolerance to the rate-decreasing effects of tianeptine but did not enhance ICSS facilitation, and there was no evidence of physical dependence. In mice, tianeptine (100 mg/kg, SC) produced naloxone-reversible respiratory depression, as well as dose-dependent and naltrexone-preventable activation of locomotor behavior. Tianeptine (100 mg/kg, SC) also significantly inhibited gastrointestinal motility.

Conclusion/Discussion: Tianeptine is a low potency but high efficacy MOR agonist that appears resistant to tolerance and dependence in our ICSS assay in rats, indicative of a lower abuse potential than commonly abused opioids. Regardless, tianeptine produced adverse effects typical of MOR agonists including depression of respiration, motor impairment, and constipation.
S39 - Identification of 11-nor- Δ -8-tetrahydrocannabinol-9-carboxylic acid in postmortem urine

Michele M. Crosby^{1,2*}, Michael T. Truver², Chris W. Chronister², Jennifer L. Hoyer², Amy M. Kinsey², Sara C. Brogan², Bruce A. Goldberger². ¹Department of Chemistry, Biochemistry and Physics, University of Tampa, Tampa, FL. ²Department of Pathology, Immunology and Laboratory Medicine, University of Florida College of Medicine, Gainesville, FL.*

Background/Introduction: The laws for growing, selling, and consuming cannabis and its related products have been changing considerably over the last few years. Δ -9-Tetrahydrocannabinol (Δ -9-THC) is the primary psychoactive constituent and the focus of cannabis research and legal debate. In 2018, the federal government legalized hemp, with hemp being defined as cannabis or a cannabis derivative containing \leq 0.3 % Δ -9-THC. This has sparked an interest in analogs of THC that can be derived from hemp and sold without oversight. One example is Δ -8-tetrahydrocannabinol (Δ -8-THC). Although less potent than Δ -9-THC, Δ -8-THC is gaining popularity and can easily be found where cannabis-related products are sold.

The forensic toxicology laboratory at the University of Florida routinely tests all cases for 11-nor- Δ -9-tetrahydrocannabinol-9-carboxylic acid (Δ -9-THC-acid) in the urine by immunoassay. Confirmation of all presumptive positive findings is performed by gas chromatography-mass spectrometry (GC-MS). A peak eluting close to Δ -9-THC-acid was identified as 11-nor- Δ -8-tetrahydrocannabinol-9-carboxylic acid (Δ -8-THC-acid).

Objectives: The objective of the study was to determine the prevalence of \triangle -8-THC-acid in postmortem casework.

Methods: Urine samples were initially screened for cannabinoids using the Thermo Scientific CEDIATM immunoassay. Subsequently, all presumptive positive samples were extracted by solid-phase extraction (SPE) and derivatized with N,O-bis(trimethylsilyl)trifluoroacetamide (BSTFA). The resulting trimethylsilyl derivative was identified using GC-MS operated in the selected ion monitoring mode (Δ -9-THC-acid: m/z **371**, 473, and 488; Δ -8-THC-acid: m/z **488**, 473, and 432). Δ -9-THC-acid and Δ -8-THC-acid have common ions, but eluted 0.04 minutes apart facilitating baseline resolution. D₃- Δ -9-THC-acid was utilized as an internal standard (m/z **374**, 476, and 491).

Results: Urine samples from approximately 900 decedents were received by the laboratory between mid-November 2021 to mid-March 2022. Presumptive positive cannabinoid immunoassay test results were observed in 194 samples. GC-MS confirmation testing was conducted, and 26 urine samples were positive for Δ -8-THC-acid. The results were as follows:

		Presumptive Positive Test Result by Cannabinoid Immunoassay	Positive Δ -8-THC-acid GC-MS Test Result	
Number of Decedents (n)		194	26	
Age (years)	Range (mean)	14-79 (43.0 years)	17-62 (36.6 years)	
	Unknown	3 (1.5%)	1 (3.8%)	
Sex	Male	157 (80.9%)	22 (84.6%)	
	Female	35 (18.0%)	4 (15.4%)	
	Unknown	2 (1.0%)	-	
Race	Caucasian	142 (73.2%)	22 (84.6%)	
	Black	39 (20.1%)	3 (11.5%)	
	Hispanic	10 (5.2%)	1 (3.8%)	
	Unknown	3 (1.5%)	-	

Six (6) of the \triangle -8-THC-acid positive cases were positive for \triangle -8-THC-acid without \triangle -9-THC-acid, and 18 samples included a history of drug and/or alcohol use. The majority of the cases were consistent with poly-drug use including fentanyl/fentanyl analogs (most prevalent), ethanol, cocaine, methamphetamine, and amphetamine.

Conclusion/Discussion: There has been an emergence of \triangle -8-THC use as indicated by the presence of \triangle -8-THC-acid in 26 of 194 presumptive positive cases for THC during a four-month period. The majority of individuals were Caucasian males with a history of drug and/or alcohol use. \triangle -9-THC-acid, as well as other drugs, were present. Given its psychoactive potential and availability, monitoring \triangle -8-THC-acid in decedents is important to characterize risk and prevalence of use.

S40 - Ohio poisoning homicides: a toxic bittersweet history

Carrie D. Mazzola*, Rindi Rico. Cuyahoga County Medical Examiner's Office, Cleveland, Ohio.

Background/Introduction: In Lucas, Ohio, lies the beautiful Malabar Farms. The former home of Pulitzer Prize winning author Louis Bromfield, and the wedding venue for Humphery Bogart and Lauren Bacall, was once the site of a horrific poisoning. Nearly 50 years before the celebrity wedding, a gristmill owner and his wife would be murdered by their own daughter, Ceely Rose.

In the last 126 years, several poisoning murders have occurred in the great state of Ohio. Known for the shores of Lake Erie, rolling farmlands, the Buckeyes, and the Rock and Roll Hall of Fame, Ohio also has a dark side. Intriguing cases of homicide by poisoning have been reported as far back as 1896 with Ceely Rose poisoning her family with arsenic. Almost 30 years later, in 1925, mysterious strychnine poisonings shocked the Ohio State University campus when two students died, and several others were sickened by "quinine/aspirin" capsules doled out at the student dispensary.

Also, in 1925, a nine-year-old Irving Sunshine was living in New York City. At that time, he did not know that he would be one of the founding fathers of modern toxicology and develop methods to solve future poisoning mysteries. Irving Sunshine moved to the Cleveland area in 1951 and served as the Chief Toxicologist for the Cuyahoga County Coroner's Office and University Hospitals, and as Professor of Toxicology at Case Western Reserve University. His cutting-edge work has enabled toxicologists to provide impeccable services and results for the forensic community.

Dr. Sunshine's work helped solve the Girts murder in Parma, Ohio. In 1992, a funeral director's wife, Diane Girts, was found slumped over in the bathtub. The forty-two-year-old woman's death initially appeared to be natural causes. After further investigation, her death was classified as a homicide due to cyanide poisoning. The poisoning was the result of her husband adding cyanide to a saltshaker she used on pasta salad the prior evening. A mere thirteen years later, Dr. Yazeed Essa also poisoned his wife with cyanide. His method was replacing the calcium in her supplements with cyanide. An international manhunt and extradition would follow.

Another popular poisoning technique is by ethylene glycol. A sweet solution that is barely detectable in sugary drinks has been used on several notable occasions in Ohio. In 2006, Matthew Podolak was being poisoned by his fiancée, over what was thought to be several weeks or even months. Holly McFeeture added antifreeze to the raspberry iced tea that he drank daily. He died in the hospital after becoming increasingly ill. Similarly, Maureen Auerswald died in 2009 after her husband staged her poisoning to look like a drunken suicide attempt. Taken to Medina County Hospital, staff was suspicious of Dennis Auerswald's story, and an investigation was launched with ethylene glycol being the poisoning culprit again.

Some poisonings may not have been intentional, but none the less a homicide while some poisonings do not lead to the direct death but are a contributing factor in the homicide. For example, in 2000, 1-year- old Allison Kuczmarski was given diphenhydramine for her naptime by her babysitter. Allison became entangled in blankets and died of asphyxiation due to the levels of diphenhydramine in her system.

While homicide by poisoning is not common, when it does occur, the toxicology community is prepared to assist in the investigation thanks to the contributions of Dr. Sunshine.

Objectives: To highlight poisoning deaths in Ohio, particularly in Cleveland and the surrounding areas; to emphasis the devotion and achievements of Dr. Irving Sunshine to the city of Cleveland and to the field of toxicology.

S41 - Fentanyl postmortem redistribution: a large population review

Erin C. Strickland* and Teresa R. Gray. Harris County Institute of Forensic Sciences, Houston, TX.

Background/Introduction: The Department of Health and Human Services declared a public health emergency due to the opioid crisis in 2017. Forensic toxicologists are asked to provide interpretation in opioid cases which can be complicated by licit and illicit uses, tolerance, and postmortem redistribution.

Fentanyl displayed average heart/peripheral blood ratios of 1.6 and 2.7 from two studies, Anderson in 2000 and Isenschmid in 2007, respectively. These references were prior to or at the cusp of the opioid epidemic and the Anderson study was limited to prescription fentanyl use. Chatterton in 2018 found a similar average ratio of 1.48. Interestingly, the heart/ peripheral blood ratio ranges increase as the study size increases. In the small 2000 study, the range was 0.7 - 4.6 (n=13), the larger 2007 study reported 0.5 - 11 (n=80), and the 2018 study reported <1 - 20.23 (n=105). Chatterton's larger and early-epidemic data set show that more current data may be necessary to better characterize the extremes possible in fentanyl postmortem redistribution.

Objectives: Review data for fentanyl collected from April 2021 to May 2022 focusing on paired blood results to evaluate PMR.

Methods: Specimens were analyzed for fentanyl using liquid chromatography mass spectrometry in multiple reaction monitoring and positive ionization mode. The analytical range for fentanyl was $0.5 - 100 \mu g/L$ in blood while norfentanyl was qualitative with a reporting limit of $0.5 \mu g/L$. Fentanyl has an uncertainty of measurement of 19% in blood at a 95.45% level of confidence. The reported results for all fentanyl-positive cases were searched from April 2021 to May 2022 and filtered for postmortem cases with quantitative results. Additionally, the fentanyl quantitative cases with paired central and peripheral blood were cross-referenced with the norfentanyl positive cases to evaluate the positivity rate.

Results: There were 360 and 271 unique cases with quantitative fentanyl results in peripheral and central sources, respectively. The fentanyl concentration range, median, and average concentrations in peripheral and central blood sources can be seen in Table 1.

	Peripheral blood	Central blood
Ν	384	295
Minimum	0.52	0.51
Maximum	260	560
Median	9.7	17
Average	15	26

Tahle 1	Fentan	I Concentrations	(11 0 /1)
Table 1.	rentany	/ COncentrations	(µg/L)

Of the fentanyl cases with quantitative results, 208 had paired central and peripheral sources (C/P) and 48 had replicates of the same source (e.g., two different tubes of femoral blood were analyzed). The C/P sources included iliac and heart (11 cases), subclavian and heart (4 cases), femoral and heart or aorta (192 cases), and femoral and chest cavity (1). Concentration ratios were calculated for replicates of the same source to rule out analytical variability contributing to C/P differences. Table 2 compares the ratios below.

s
)

	Central/Peripheral	Same Source	
N	208	208 48	
Minimum	0.25	0.15	
Maximum	timum 17.10 1.45		
Median	1.61	1.00	
Average	2.32	0.97	

The C/P ratios did not appear to have a significant correlation with fentanyl concentration or postmortem interval. It is noteworthy that when norfentanyl was not detected in either sample there was a trend towards higher C/P ratios.

Conclusion/Discussion: The average C/P ratio for our large data set is consistent with previous reports. The range of C/P ratios (0.25 - 17.10) confirms the Chatterton observation that the postmortem distribution of fentanyl may be more varied than previously thought. Concentration ratios in replicates from the same source confirm that the observations of PMR are real. Having two larger data sets that are early epidemic and later epidemic also shows that there may be a difference in the C/P ratios observed in illicit related fentanyl deaths compared to prescription fentanyl related deaths. With the increase in fentanyl-related deaths, toxicologists and pathologists should be mindful of postmortem redistribution when interpreting fentanyl concentrations.

S42 - Babies and drugs: drug prevalence in Miami-Dade County infant deaths

Jennifer Gonyea*, George W. Hime, Diane M. Boland. Miami-Dade Medical Examiner Department, Miami, FL.

Background/Introduction: The world is in the midst of a drug crisis and, unfortunately, children are not exempt. In infant deaths, drugs identified are often legitimate prescriptions or over-the-counter medications due to illness; however, there are postmortem cases in which illicit substances are identified. Infants may have some passive exposure to an illicit substance through dermal contact, secondhand smoke inhalation, or exposure via breastmilk or placenta, if in utero. Intentional poisonings and accidental ingestion can also occur and should be of no surprise given the drug epidemic in the United States. In Miami-Dade County, one infant was discovered to have eutylone, a synthetic cathinone, in their system.

Objectives: The objectives of this presentation are to offer an overview of infant deaths in Miami-Dade County from 2012 to present and the corresponding manners of death, to examine drugs present in several of those cases, and to highlight a case of eutylone detected in an infant.

Methods: A query of Miami-Dade County Medical Examiner cases was conducted for infant deaths under 2 years of age from 2012 to present. The cause of death for each case with the corresponding drugs present were organized and tabulated according to manner of death. The drugs present were detected during routine toxicology testing with confirmation and quantitation performed as needed.

Results: A total of 259 infant deaths, under 2 years old, occurred from 2012 to present in Miami-Dade County. The manners of death determined were accident, homicide, natural, fetal death, undetermined, and pending. Most of the accidents were due to asphyxia due to unsafe sleep environments. Homicides involved trauma either to the infant or to the mother first, then subsequently, the baby. Natural deaths ranged from prematurity to COVID-19. Fetal deaths involved either maternal trauma such as from a motor vehicle collision or fetal trauma such as placental insufficiency. When no apparent cause and manner of death was determined, the case was reported as undetermined and when the case is waiting on supplemental information, it was reported as pending. Of the 259 cases studied, 32% (83 cases) had drugs present; the drugs identified ranged from traditional over-the-counter medications such as acetaminophen and diphenhydramine to illicit substances such as cocaine and eutylone. In most of the positive cases, the drugs were considered incidental and not included in the cause of death based on the medicolegal investigation. For these cases, the drugs were typically not quantified due to limited sample volume or quantification was deemed unnecessary for cause and manner of death determination. Two out of the 83 cases listed cocaine in the cause of death, however only as a contributing factor and not as the sole factor in death. Another particular case involved a 2-month-old infant presenting with 12 ng/mL of eutylone in the chest fluid who died under other suspicious circumstances as well, including a lung infection, a genetic disorder, an unsafe sleep environment, poor parental judgement, potential neglect, and inconsistent stories. This overall uncertainty surrounding the terminal event led to an undetermined cause and manner of death.

Conclusion/Discussion: An analysis of infant deaths in Miami-Dade County over the last 10 years is presented along with causes and manners of death and drugs detected. There were many cases with medicinal drugs present; however, illicit substances were also identified which can often complicate the cause and manner of death determination. At the Medical Examiner Department, all aspects of the case are under scrutiny, not just the toxicology. Consequently, the infant death involving eutylone was ruled as undetermined for both cause and manner of death due to the extenuating circumstances surrounding the death.

S43 - Missed opportunities in medicolegal drug death investigations

*Sara J. Schreiber MS^a, Brian L. Peterson MD^a

^aForensic Toxicology Laboratory, Office of the Medical Examiner, Milwaukee, WI.

Background/Introduction: Medicolegal death investigation (MDI) is an area of forensic science that carries a unique responsibility. Scientists in this field investigate deaths that are sudden, unexpected, or unattended and certify cause (COD) and manner of death (MOD). In response to the increase in deaths, including drug-related deaths, offices make policy decisions for case management. Case volume can be mitigated by reducing full forensic autopsies. This is accomplished by releasing decedents to a funeral home with certification determined after medical record review, or by externally examining decedents and collecting specimens for toxicology. Drug-related deaths cause are increasing at a rate hard for offices to keep pace with, especially given the shortage of practicing forensic pathologists.

Policy choices to limit or eliminate testing in certain cases creates risk that cause of death may be inaccurately certified. A drug-related cause will be missed without toxicology analysis. There is little standardization in this area of MDI resulting in a wide array of policy and practice.

Objectives: This study provides evidence-based support for addressing policies decisions identifying the cases that should have a full toxicology screen to accompany the death investigation even without an autopsy.

Methods: The laboratory information system of the Milwaukee County Medical Examiner's Office (MCMEO) was queried for cases from 2020 and 2021 that were designated as a "body release" for which the cause of death was natural, and blood was available for testing. 326 cases were identified for inclusion. The blood samples were subclavian blood in grey top tubes that had been frozen since collection. The time from collection to analysis varied throughout the sample population. The blood samples were screened by liquid chromatography-quadrupole mass spectrometry with a time-of-flight detector. Approximately 800 analytes were targeted. The analytes detected were evaluated for ones frequently contribute to COD such as opioids, stimulants, and illicit substances. Appropriate confirmatory analysis was performed with results reported to the forensic pathologist for evaluation. Death certificates were amended where appropriate.

Results: As a result of the toxicology analysis, 18 cases were identified where the COD and MOD was amended to a drug-related cause. One case was changed to suicide after a conversation with the family about the toxicology findings. The remaining 17 cases were changed to an MOD of accident. The patient demographics where the death certificate was changed were 13 males (age from 43 to 70, average 57 years) and 5 females (age from 52-72 years, average of 60 years). The original COD in these cases were primarily cardiovascular in nature, n=10, two were complications of diabetes mellitus, and 5 were chronic obstructive pulmonary disease. After the toxicology analysis, the COD was amended to cocaine intoxication, n=8, and opioids (fentanyl, heroin, oxycodone, methadone, acetyl fentanyl, fluorofentanyl) n=10 and each had an MOD of accident. One case had the death attributed to venlafaxine and was ruled a suicide.

Conclusion/Discussion: As an office creates policy for determining which case will receive a full forensic autopsy, they must also evaluate the risk associated with that decision. This study provides evidence-based data to show that the MCMEO was underrepresenting drug-related deaths. Natural disease can be due to chronic drug use consequently these cases need careful consideration. Offices should strongly consider the added value toxicology results can bring to a death investigation.

S44 - Analytical challenges associated with the quantitative analysis of tetrahydrocannabinol isomers in biological matrices

Denise Herr, PhD; Ali Siddiqi; Rebecca Wagner, PhD*. Virginia Department of Forensic Science, Richmond, VA.

Background/Introduction: The ever-changing climate of cannabis decriminalization/legalization has had a significant impact on forensic testing laboratories. Traditionally, changes within the seized drug community can be utilized to anticipate analyses needed within forensic toxicology laboratories. The confirmation and quantitation of Δ^9 -tetrahydrocannabinol (Δ^9 -THC) and its metabolites in biological matrices is commonplace amongst testing laboratories. Forensic toxicology laboratories have to adapt their methodologies to the legal consumption of manufactured cannabis products containing other phytocannabinoids and isomers of Δ^9 -THC.

The Toxicology Section of the Virginia Department of Forensic Science (DFS) evaluates biological specimens for the presence of drugs in criminal matters, including drug and/or alcohol impaired driving (DUI/DUID) and death investigations. Over the past five years, DFS has observed a 25% increase in the reporting of quantitative values for Δ^9 -THC. Recently, DFS Toxicology has detected the presence of Δ^8 -THC in casework specimens. Given the current climate of tetrahydrocannabinol isomers and legislative language surrounding cannabinoids, traditional methods require additional development to effectively separate, confirm, and quantify not only Δ^9 -THC but its commonly observed isomers including Δ^8 -THC, $\Delta^{6a,10a}$ -THC, and Δ^{10} -THC.

Objectives: The objectives for the project are: 1) expand the scope for cannabinoid testing in forensic toxicology laboratories to include phytocannabinoid constituents and metabolites that are used in consumer products; and 2) investigate commercially available extraction substrates and instrumental conditions to increase selectivity and mitigate ionization suppression commonly encountered in the analysis of cannabinoids in biological matrices.

Methods: An Agilent Technologies 1290 Infinity liquid chromatograph coupled to either a 6430, 6460, or 6470 quadrupole mass spectrometer was utilized for the instrumental method development. A Poroshell 120 EC-C18 3.0 x 50 mm column with a 2.7 µm particle size column from Agilent Technologies was utilized alongside a mobile phase composition of water fortified with 0.1% formic acid (Mobile Phase A) and 80:20 methanol:acetonitrile (Mobile Phase B). A gradient elution was employed over a total run time of 11 minutes for chromatographic resolution of THC isomers.

Sample preparation is critical in the development of an analytical method encompassing antemortem and postmortem specimens. This is increasingly important for cannabinoids analysis where ionization suppression from high lipid content can significantly impact the analytical result. Three sample preparation methods were developed and compared to determine the optimal sample preparation technique. These methods included a traditional liquid-liquid extraction employing 9:1 n-hexane:ethyl acetate, a solid phase extraction (SPE), and a supported liquid extraction (SLE). The lower limit of detection, recovery, ionization suppression/enhancement, and basic chromatographic characteristics were utilized to compare the sample preparation methods. Each method was assessed at concentrations delineated for cannabinoids within the ANSI/ASB scope and sensitivity standards.

Results: Although the liquid-liquid extraction is a common sample preparation utilized in cannabinoids analysis, it suffers from ionization suppression in whole blood specimens. The SPE procedure is a multi-step extraction that includes a protein precipitation prior to the addition of a specimen onto the solid phase extraction column. This lengthy procedure is not ideal for laboratories that have a high throughput of cannabinoids analysis. The SLE provides reduced ionization suppression compared to the liquid-liquid extraction. Additionally, it provides a sample preparation procedure that simply acidifies a specimen prior to loading on the SLE cartridge followed by elution with 3.0 mL of ethyl acetate and 3.0 mL of n-hexane.

Conclusion/Discussion: An extensive comparison of sample preparation is critical in the development of methods regarding cannabinoids in whole blood specimens. The SLE procedure outperformed the liquid-liquid extraction and SPE while also being amendable to automation in the future. The comparison of methods enables a data driven decision for the most effective method for a laboratory from an efficiency and analytical perspective.

S45 - Changing landscape of marijuana products as evident from biological sample testing.

Eva Reichardt¹, G. Brent Dawson², Tara Arends² and **Sumandeep Rana^{3*}**. ¹Abbott, Abingdon, Oxfordshire, United Kingdom; ²Redwood Toxicology Laboratory, Inc, California, Santa Rosa;³Abbott, Chicago, Illinois.

Introduction: There are reports of increased prevalence of Delta-8-THC, a psychoactive cannabinoid produced in small quantities by *Cannabis Sativa*. Extracts and dabs enriched with Delta-8-THC are marketed as a legal alternative to Delta-9-THC. While its effects are similar to that of Delta-9-THC, it is reported to be less potent with milder psychoactive effects.

In 2020, the DEA issued an interim rule to link the Farm Bill with the Controlled Substances Act to ensure that all synthetically derived tetrahydrocannabinols remain schedule 1 controlled substances¹. Furthermore, the FDA issued guidance around the public health risk linked to Delta-8-THC². Due to the risks, Delta-8-THC is now regulated, restricted or banned in at least 20 states in the US.

Objectives: We first reported on Delta-8-carboxy-THC in urine samples and its implications to the drug testing programs at TIAFT 2019. In this study, we evaluated the current prevalence of Delta-8-carboxy-THC in human urine samples from court-ordered monitoring programs in the U.S and compared to 2019 data to assess changes in prevalence and potency.

Methods: 10µL of internal standard, 15µL of 10X buffer, 5µL of IMCS Enzyme RT and 135µL of deionized water was added to 50µL sample aliquot. After 15 minutes, 185μ L of 0.1% formic acid with 2mM ammonium formate in 50:50 water: acetonitrile solution was added and vortexed for 1 minute before centrifugation for 20 min at 3750 rpm. 200µL of the supernatant was added to the autosampler plates and analysis performed with 1 µL injection. Pipetting and vortexing steps was performed using Hamilton Microlab Nimbus HD or Hamilton Microlab Nimbus 96 Workstations. A multiplexed Shimadzu Prominence HPLC system with a Sciex 4000 Qtrap was used in negative mode ionization with two transitions monitored for Delta-8-carboxy-THC metabolite (343.2 > 245.2 and 343.2 > 191.1) and one transition monitored for the deuterated internal standard (352.2 > 308.2). Mobile phases were 0.01% formic acid in D.I. water (A) and 0.01% acetic acid in methanol (B). The 5 minute gradient started at 70% B and ramped to 75% B over 3.5 minutes and then to 100% B over 0.1 minutes where it was held for 1 minute prior to a 0.5 minute re-equilibration at 70% B. The analytical column was a 50 x 4.6mm 2.7µm Restek Raptor FluoroPhenyl with a 0.5µm IDEX filter Guard column.

Results and Discussion: Samples previously analyzed in 2019 showed Delta-8-carboxy-THC was found in 3% of the specimens (n=1100). All of the samples also contained the Delta-9-carboxy-THC metabolite and, in all except four samples the concentration of Delta-9-THC metabolite was higher than Delta-8-carboxy-THC.

In comparison, 45% of the samples from three randomly tested batches from 2022 (n=287) contained Delta-8-carboxy-THC. Delta-9-carboxy-THC was found in all positive Delta-8-carboxy-THC samples except two. In samples that contained both analytes (n=128), 7.8% (n=10) had Delta-9-carboxy-THC concentrations below the SAMHSA reporting cutoff of 15ng/mL. Delta-8-carboxy-THC concentrations in these samples far exceeded the cutoff. Further, Delta-8-carboxy-THC concentration in 22% (n=29) of the samples exceeded that of Delta-9-carboxy-THC.

This study shows the prevalence of Delta-8-THC in the United States has increased significantly between 2019 and 2022, rising from 3% to 45% in the positive samples, respectively. This may be a particular risk in states where Delta-8-THC is marketed as a legal alternative to Delta-9-THC. Further, the concentrations of Delta-8-carboxy-THC in the positives have increased. Unless Delta-8-THC is monitored in the testing profile, positive samples will go undetected.

Conclusions: Prevalence and concentrations of Delta-8-carboxy-THC in court-ordered urine specimens has increased significantly over the last 3 years. The data from this study is consistent with data recently reported by RTI³ and further highlights the need for laboratories to include Delta-8-carboxy-THC in their testing methodologies.

S46 - Development and optimization of a large urine antipsychotic drug panel and observations of clozapine internal standard enhancement

Theresa M. Meli^{1*}, Wen Dui¹, Karin O. Thomassian², Anita Dermartirosian², Victor E. Vandell², Michael P. Smith¹, Leslie E. Edinboro^{1,3} and Sarah H. Bartock^{1.} ¹Quest Diagnostics Nichols Institute, 14225 Newbrook Drive, Chantilly, VA 20151; ²Quest Diagnostics, 27027 Tourney Road, Valencia, CA 91355;³Current affiliation: Edinboro Consulting, LLC, 10501 Delray Road, Glen Allen, VA 23060.

Background/Introduction: Antipsychotic drugs are highly prescribed in the United States. These drugs are commonly used to control symptoms of anxiety and mania, although off-label prescription uses also occur. Antipsychotic drug assays have historically been comprised of a small, targeted number of drugs rather than large panels. Larger panel testing that simultaneously measure a broader array of drugs using definitive liquid chromatography-tandem mass spectrometry (LC-MS/MS) based testing is a means of establishing baseline drug use (prescription and non-prescription) in some patients. However, metabolic processing such as the reported glucuronidation of some antipsychotic drugs, could potentially impact their accurate quantitation in urine and mislead clinical assessment of the patient's drug use.

Objectives:The development and optimization of a large definitive urine antipsychotic drug and metabolite panel using enzymatic hydrolysis and extraction by dispersive solid-phase extraction with disposable pipette extraction (DPX) tips and analysis by LC-MS/MS.

Methods:LC-MS/MS analysis for 18 antipsychotic drugs and 8 metabolites was performed on an AB Sciex 4500 Q-Trap mass spectrometer with an electrospray ionization source. The mass spectrometer was interfaced with a Sciex Exion LC system. A Phenomenex 2.6 μ Phenyl-Hexyl 50×4.6mm column was used with aqueous mobile phase consisting of 10mM ammonium formate in water and organic mobile phase consisting of 75:25 methanol:acetonitrile (v/v). Matched deuterated ISTDs were used for 16 of 26 analytes. Calibration and QC material were prepared in drug-negative urine using diluted certified reference material stocks. Amitriptyline, an antidepressant, was added to the calibration curve to monitor hydrolysis QC efficiency. No certified reference material for any glucuronidated antipsychotic drug is available currently. Sample preparation utilized DPX strong-cation exchange tips and Kura B-One[®] (β -glucuronidase) enzyme. The analytes in this panel included:

Aripiprazole	Haloperidol	Risperidone
Dehydroaripiprazole	Loxapine	9-Hydroxyrisperidone
Brexpiprazole	8-Hydroxyloxapine	Thioridazine
Cariprazine	Lurasidone	Thiothixene
Chlorpromazine	Mesoridazine	Trifluoperazine
7-Hydroxychlorpromazine	Molindone	Ziprasidone
Clozapine	Olanzapine	Amitriptyline (Not Reported)
Norclozapine	Desmethylolanzapine	
Fluphenazine	Quetiapine	
7-Hydroxyfluphenazine	Norquetiapine	

Results/Discussion: Limits of quantitation (LOQs) ranged from 0.5 ng/mL to 25 ng/mL with an upper limit of 500 ng/mL for all analytes. Linearity was achieved with a quadratic $1/x^2$ weighting. Between-run imprecision (n=25) for 3 QCs ranged from 2% to 10%. Overall extraction efficiencies were 66% to 108% and matrix effects were within ±25%. No assay carry-over was observed. No interferences were observed from addition of 5,000 ng/mL of >150 compounds (commonly seen prescription and non-prescription drugs) to the samples. Several important observations were made during validation. Between-run imprecision was 4% (n=25) with β -glucuronide hydrolysis, which was an essential step in extracting patient specimens. Of 26 analytes, 10 showed a substantial increase in calculated concentration with enzymatic hydrolysis. The ISTD clozapine-D4 transition chosen for this panel was initially m/z 331 \rightarrow 272, based on previous literature. However, enzymatic hydrolysis showed signal enhancement of this ISTD transition in random deidentified clozapine-positive patient specimens. Consequently, an unexpected decrease in calculated concentration was observed that did not follow the expected hydrolysis trend. This trend persisted even with various types of enzyme (IMCS IMCSzyme[®], Kura B-One[®], and Kura

BG Turbo[®]). In contrast, no effect was observed on the ISTD signal in either clozapine-negative specimens or clozapine-positive specimens without hydrolysis. The unexplained aberrant ISTD signal enhancement was eliminated by changing the clozapine-D4 transition to m/z 331 \rightarrow 273. Deidentified clozapine-positive patient specimen discards were retested using the new ISTD transition and results portrayed the anticipated increase when using hydrolysis.

Conclusion:The use of this quantitative 26-analyte antipsychotic urine drug panel offers detection of a wider range of analytes to better serve various patient and client populations. Sample preparation with enzymatic hydrolysis provides a more accurate representation of total antipsychotic urine contents.

S47 - The SOFT professional mentoring program: forward momentum

James M. (Jim) Burris¹, Amanda Cadau^{*2}, Marisol Castaneto³, Marta Concheiro-Guisan⁴, Lindsay Glicksberg⁵, **Helen H. Ha**⁶, Erin L. Karschner⁷, Graham Kennedy⁸, Afton Martinez⁹, Beth Olson¹⁰, Michelle R. Peace¹¹, Kim L. Samano¹², Dawn Sklerov¹³, Courtney M. Wardwell¹⁴.

¹Central Texas Forensic Toxicology, Austin, TX. ²New York State Police, Albany, NY. ³DOD Food Analysis and Diagnostic Laboratory, Joint Base San Antonio, Fort Sam Houston, TX. ⁴John Jay College, City University of New York, New York, NY. ⁵Southwestern Institute of Forensic Sciences, Dallas, TX.

⁶ Orange County Crime Lab, Santa Ana, CA. ⁷ Armed Forces Medical Examiner System, Dover AFB, DE.

⁸ United States Drug Testing Laboratories, Inc., Des Plaines, IL. ⁹Henderson Police Department, Criminalistics, Henderson, NV. ¹⁰Society of Forensic Toxicologists, Mesa, AZ. ¹¹Department of Forensic Science, Virginia Commonwealth University, Richmond, VA. ¹²Johnson County Medical Examiner's Office, Olathe, KS. ¹³Washington State Patrol Toxicology Laboratory, Seattle, WA. ¹⁴Virginia Department of Forensic Science, Manassas, VA.

Background/Introduction: The goals of the SOFT Professional Mentoring Program (PMP) are to develop and nurture future leaders of the organization and provide a forum for one-on-one career advice and mutually beneficial knowledge transfer to support and advance the organization and forensic toxicology practice. The PMP committee was founded in 2019, launched in 2020 and currently has fourteen members. Program subcommittees include: Strategic Planning, Incoming Class, Programming and Resources. Participant surveys assess committee-defined mentoring benefits, track progress between pairs, establish metrics and target program improvements.

Objectives: This presentation will describe self-reported mentor/mentee program outcomes and discuss the maturation of the PMP between 2020 and 2021.

Methods: In addition to one-on-one mentoring sessions, the 2021 program offered other activities based on the 2020 participants' feedback, including a kick-off event, webinars, monthly resources emails and a mentoring activity at SOFT-YFT (Young Forensic Toxicologists) Program. Feedback on participant achievements (goals assessment), and program structure and content (program value assessment) were collected via participant surveys in 2020 and 2021. Similar questionnaires were provided both years; however, the 2021 assessment limited open-ended questions to facilitate data analysis and did not focus on COVID-19 impact.

Goals assessment questions included: career advancement, engagement within SOFT, data/case/research dissemination, interpersonal/leadership skills, and technical development. Program value assessment questions included: engaging with the SOFT mission, encouraging voices, time management, career coaching, professional accountability, knowledge transfer, growth of talent to innovate the field, leadership development, legacy building, general learning and expanding professional network.

Results: Seventy-four people registered in the 2020 inaugural year, and 84 in 2021. The total number of pairs increased from 41 in 2020 to 51 in 2021 (6 pairs continued from 2020 and 45 new pairs were created with new and existing participants; mentors also may serve more than one mentee). Assessment responses were received from 68% of participants in 2020 and from 76% in 2021. In both years, the program met the participants' original expectations (88% in 2020 and 90% in 2021). In 2021, 80% of participants did not change their goals throughout the year, compared to 43% in 2020 during COVID-19. For professional goals, the most significant outcome was "SOFT engagement" in 2020 in which more than 50% of participants responded that they 'met and somewhat/significantly exceeded their expectations.' In 2021, the most significant outcomes were "career advancement" and "development of interpersonal/leadership skills." Participants were overall more satisfied with the benefits they obtained from the program were encouragement (73%), transfer of knowledge (75%) and expansion of their professional network (80%). In 2021, those same benefits increased to 88%, 96% and 85%, respectively. In addition, the majority of respondents (>70%) highlighted increased engagement with SOFT (76%), career coaching (81%), professional accountability (74%), growth of talent/innovation (87%), leadership development (81%) and general learning (73%). Among the new activities organized in the 2021 program, the most valued (73-84% high or moderate value) were the kick-off event, webinars by external speakers, and monthly mentoring resource emails.

Conclusion/Discussion: The formal SOFT mentoring program has continued to grow and mature since its inception in 2019. Feedback from the annual assessment data has strongly demonstrated the value of a formal mentorship program to the SOFT organization. Modifications based on participant feedback remain critical to the program's evolution. Both participation and increased positive outcomes were reported from 2020 to 2021, which further supports the maturation of the PMP and continued evolution based on directed participation feedback and strategic planning.

S48 - Standards development activities in forensic toxicology

Marc A. LeBeau, PhD, F-ABFT*, FBI Laboratory, Quantico, VA 22135 .

Background/Introduction: The development, implementation, and awareness of standards has been rapidly increasing throughout all the forensic science disciplines.

Objectives: This presentation will update the forensic toxicology community on the status of standards development activities; to include ASB-published American National Standards, the ASB toxicology standards that have been placed onto the OSAC Registry, and documents in the draft stage at both ASB and OSAC. Additionally, a number of tools and resources are under development through an AAFS-NIST cooperative agreement and the latest information on these tools will be discussed. These tools and resources include free technical trainings, factsheets that help educate stakeholders on the purpose and benefits of these standards, and checklists to assist laboratories conduct gap analyses and conformance monitoring in their implementation of these standards.

S49 - Synthetic cannabinoids in human performance and postmortem casework from 2019-2022

Erin L. Karschner*, Joshua Z. Seither, Sarah Shoemaker, Jeffrey P. Walterscheid. Division of Forensic Toxicology, Armed Forces Medical Examiner System, Dover AFB, DE, USA.

Background/Introduction: Synthetic cannabinoids are novel psychoactive substances (NPS) that became popular for their cannabimimetic effects and their inability to be detected by traditional drug testing. This class of compounds is ever-changing and creates a moving target for forensic toxicology laboratories. In July 2021, China instituted a class-wide ban on the production and sale of synthetic cannabinoids. As a result, manufacturers developed new compounds that circumvent existing legislation and avoid detection under current analytical methods. The U.S. military's counternarcotics mission was designed to promote the health, safety, and readiness of military service members through fit-for-duty and investigative drug testing. This mission can also provide a unique window into clinical toxicological aspects of emerging substances when controlled studies are lacking.

Objectives: This presentation will describe synthetic cannabinoid trends from 590 human performance and postmortem cases over the last four years, with a focus on the impact of the class-wide synthetic cannabinoid ban in China. Common effects and indicators observed in these cases will also be discussed.

Methods: Specimens were submitted for toxicology testing between 2019 and 2022. Testing was assigned depending on case history or request. Synthetic cannabinoids were screened by a targeted liquid chromatography tandem mass spectrometry (LC-MS/MS) assay until April 2020 when screening was moved to a liquid chromatography quadrupole time of flight mass spectrometry method. All presumptive positives were qualitatively confirmed by LC-MS/MS at detection limits ranging from 0.1 to 1.0 ng/mL and all methods were validated to ASB Standard 036.

Results: From January 2019 to June 2022, the Division of Forensic Toxicology processed 590 human performance and postmortem cases involving synthetic cannabinoids. Of these cases, 516 were from general investigative or fit-for-duty incidents and 30 were from unit sweeps. Additional positive cases were from more traditional case types such as driving while intoxicated (31), drug facilitated sexual assault (4), and postmortem (9). From 2019 to 2020, positive cases increased from 185 to 249 per year but fell to 140 in 2021. Case numbers continued to decrease in 2022, with only 16 reported positive cases as of June. Overall, the most common cognitive and behavioral effects included the appearance of intoxication, impaired or slurred speech, confusion, impaired motor skills, sluggishness or drowsiness, delayed responses, and loss of consciousness. The top physiological indicators included glassy/watery bloodshot eyes, vomiting, seizures or shaking, and diaphoresis. The most common administration method was vaping.

Conclusion/Discussion: Forensic toxicology laboratories are charged with maintaining a relevant testing scope, even as the number of NPS continues to increase. Analytically-confirmed synthetic cannabinoid cases have decreased after the class-wide ban. However, cases demonstrating a history of vaping and many of the same symptoms as the previous generation synthetic cannabinoids are still observed. This emphasizes the importance of elucidating the structures of new synthetic cannabinoids that do not meet the Chinese regulatory definition, determining appropriate analytical targets, and updating screening and confirmation panels. In addition to the class-wide ban, the rising popularity of phytocannabinoid analogs and derivatives (e.g., Δ^{8} -THC, Δ^{10} -THC, THC-O acetate, tetrahydrocannabiphorol, tetrahydrocannabihexol, tetrahydrocannabinoid cases. In-dividuals, particularly in the military population, may be seeking the use of substances they believe will elude traditional drug testing panels.

S50 - A snapshot in time—2018-2021: rise and fall of substances by monitoring NFLIS-Drug

DeMia Pressley,^{*1} Will Heuett,¹ Terrence Boos,¹ Agnes Winokur, ¹ Katherine Bollinger,² Megan Grabenauer,² BeLinda Weimer,² David Heller,² Jeffrey Ancheta,² Neelima Kunta,² Jeri Ropero-Miller,² Hope Smiley-McDonald². ¹U.S. Drug Enforcement Administration, Springfield, VA. ²RTI International, Research Triangle Park, NC.

Background/Introduction: The National Forensic Laboratory Information System (NFLIS) is a program of the Drug Enforcement Administration, Diversion Control Division. NFLIS-Drug systematically collects drug identification results from case submission to Federal, State, and local forensic laboratories that analyze controlled and noncontrolled substances secured in law enforcement operations. In 2019, NFLIS-Drug started publishing quarterly Snapshots as a mechanism to provide more timely data to the community. These Snapshots include new drugs not previously submitted to NFLIS-Drug, highlights of the top 5 substances in specific drug classes, and noteworthy upward trends of substances. Another addition to NFLIS includes a collaborative effort between two DEA programs to create a practitioner-driven communication platform known as the NFLIS DEA Synth-Opioids Real-Time Communication Network (Synth-Opioids). Another resource is the public Data Query System (DQS).

Objectives: To better understand emerging substances presented in the Snapshot reports, specifically those that persisted over time, and provide information on two additional NFLIS resources for monitoring emerging substances: Synth-Opioids and DQS.

Methods: We created a comprehensive list of newly reported substances from all Snapshot publications from 2019 through 2021. This presentation includes these data and any related isomers from January 1, 2018, to December 31, 2021. The emerging substances included are synthetic cannabinoids, fentanyl-related compounds, synthetic cathinones and other narcotic analgesics. Annual and regional trends for these substances reported by Federal, State, and local laboratories are presented. Selected upward trends of substances of interest and reports of selected substances by State are presented. The impact of Synth-Opioids and the public DQS will be highlighted.

Results: Since 2019, Snapshots have reported over 70 new substances of interest to the community. The data analyzed include those substances and their positional isomers. This resulted in over 100 substances from 13 drug categories and 50 states and the District of Columbia. Between 2018 and 2021, there were over 50,000 drug reports of the selected substances. Of those substances, close to 30,000 drug reports were in 2021. Almost half of the drug reports of the selected substances were fentanyl-related compounds and synthetic cannabinoids. The top 10 most frequently reported substances between 2018 and 2021 were fluorofentanyl, MDMB-4en-PINACA, ADB-BUTINACA, fluoroisobutyryl fentanyl, phenethyl 4-ANPP, metonitazene, fluoro-EDMB-PINACA, fluoro-EMB-PICA, and fluoro-MDMB-BUTICA. When more than one isomer was reported, synthetic cannabinoids were more likely to be reported as a specific isomer whereas fentanyl related substances were more likely to be reported as an undetermined isomer.

Synth-Opioids brings together over 500 members from 11 countries to communicate about identifying new substances, analytical challenges, policies, and practices. In 2021, members helped identify 11 unknown substances and assisted with posts related to information on increases in substances identified in laboratories and requests for spectra. Topics from the forum resulted in three formal new drug announcements. NFLIS has added a publicly available DQS to the NFLIS website. Once logged into the website, data can be viewed by drug class, substance name, and region, and can be aggregated to annual, quarterly, or monthly totals. An enhanced feature is the ability to export query results to excel and a built-in data visualization tool to export specific graphics of the data complete with an appropriate citation for reproducibility.

Conclusion/Discussion: Many of the substances that emerged between 2018 and 2021 also subsided within that time, however several have persisted. NFLIS offers several resources to help forensic toxicologists effectively monitor and analyze for emerging substances. The quarterly snapshot publications list newly reported substances reported to NFLIS. The DQS allows near real-time access to NFLIS data. The Synth-Opioids discussion forum provides a mechanism for communication between professionals to assist with identification of unknowns and general information exchange.

S51 - Nitazenes are coming to town: development of a qualitative screening method for benzimidazole opioids in postmortem specimens using LC-QQQ-MS/MS

Elisa N. Shoff*, Joseph H. Kahl, George W. Hime, and Diane M. Boland.

Miami-Dade Medical Examiner Department, Miami, FL.

Background/Introduction: At the Miami-Dade Medical Examiner Department (MDME), the rate of poly-drug toxicities has more than doubled in the last two years due to an increase in illicit fentanyl use. However, in the past 8 months, a pattern has emerged of suspected drug overdose-related deaths either screening negative for drugs, or containing minimal, non-fatal levels of otherwise toxic illicit substances. With the recent emergence of new synthetic opioids, and the detection of two of these benzimidazole opioids (Metonitazene, Protonitazene) in a drug paraphernalia case sample, the need for a targeted analysis for these compounds became imperative. Due to the potency and subsequent low concentrations in whole blood of these compounds, a basic drug screen method by gas chromatography-mass spectrometry (GC-MS) was not sensitive enough to identify these substances in postmortem casework.

Objectives: The objective of this work was to validate a targeted qualitative screening method for 10 novel benzimidazole opioids in postmortem samples, and to demonstrate the methods impact on postmortem detection of potent nitazene substances.

Methods: A Shimadzu Nexera X2 ultra-high performance liquid chromatograph (UHPLC) coupled to a Shimadzu 8060 triple quadrupole tandem mass spectrometer (QQQ-MS/MS) was validated to screen for 10 novel benzimidazole opioids relevant to current NPS trends. UHPLC separation was achieved by using a gradient elution on a Restek Raptor[®] Biphenyl column (50 x 2.1 mm, 2.7 µm, 90 Å) with an aqueous mobile phase of 0.1% formic acid in water, and an organic mobile phase of methanol at a 75:25 starting ratio. The QQQ-MS/MS was operated using electrospray ionization (ESI) with positive polarity. Data was collected using multiple reaction monitoring (MRM) with a minimum of three targeted transitions for each analyte. Positive identification was achieved using a known retention time, and the detection of all appropriate transitions at the accepted ion ratio allowances. Prior to analysis on the instrument, compounds were extracted from blood, brain, liver, serum, and urine using UCT CleanScreen mixed-mode solid-phase extraction columns.

Results: Separation of all 10 compounds, including isomeric pairs, was achieved within a 3.5-minute collection window, with a total runtime of 8.25 minutes. The limit of detection was administrative set at 0.05 ng/mL for all analytes. Endogenous interferences from decomposed matrices and exogenous interferences from commonly detected analytes were found to be insignificant. Extracts were stable in the refrigerated autosampler for 72 hours. No significant ionization suppression/enhancement was observed.

Conclusion/Discussion: This method has proven to be invaluable for postmortem casework where drug paraphernalia and/or case history suggest the presence of the targeted compounds, but corresponding analysis of the case samples was negative by GC-MS. Traditionally, such a method would be developed on our LC-Ion Trap-MS, however instrument limitations with these compounds was discovered. Over 60 select postmortem MDME cases were analyzed using this method since March 2022. Most of these cases were pending toxicological findings, and after all routine screening procedures on both GC-MS and LC-Ion Trap-MS, they still had an undetermined cause of death. So far, 5% of these cases were positive for benzimidazole opioids, specifically, Metonitazene, N-Pyrrolidino Etonitazene, and Protonitazene. More cases are expected to be positive as we expand our scope of screening to include most pending toxicology cases.

S52 - Fluorofentanyl detection by LC-QToFMS and prevalence in postmortem toxicology

Kevin G. Shanks, M.S.*, Stuart A.K. Kurtz, M.S., and George S. Behonick, PhD. Axis Forensic Toxicology, Indianapolis, IN 46268.

Background/Introduction: Novel psychoactive substances (NPS) have been an emerging problem in the United States for decades, but over the last several years, novel opioids have predominated and caused most of the morbidity and mortality associated with these trending drugs. Fentanyl analogs were prevalent in the United States from 2013-2018, but in 2018, the United States Drug Enforcement Administration (DEA) scheduled all "fentanyl-related substances" as Schedule I controlled substances. As expected, the vast majority of fentanyl analogs disappeared to only be replaced by non-fentanyl-related NPS opioids (i.e. the nitazene family). However in late 2020-early 2021, a new-ish fentanyl analog – fluoro-fentanyl – was reported by NPS Discovery and the DEA's Emerging Threats Reports as increasing in prevalence in the US. Fluorofentanyl has three positional isomers – meta (*m*), ortho (*o*), and para (*p*).

Objectives: Due to reports of the recent increase of fluorofentanyl in the US, in June 2021, we added fluorofentanyl (non-isomer specific) to our scope of comprehensive testing in postmortem blood samples. The objective of this study is to describe the prevalence of fluorofentanyl in our toxicology casework for 06/01/2021 - 05/01/2022, along with the other substances it is detected alongside. Cause of death determination as opined by the medical examiner is not included in this study. A portion of this information was presented in a SOFT Continuing Education Webinar in June 2022.

Methods: Blood specimens were drawn by the medical examiner at autopsy, collected in vials containing sodium fluoride as a preservative, and sent to the laboratory for comprehensive toxicological analyses. Screening procedures included a targeted comprehensive screen for drugs of abuse, prescription drugs, over-the-counter agents, and novel psychoactive substances by liquid chromatography with quadrupole time of flight mass spectrometry (LC-QToF-MS) and volatile analysis by head space gas chromatography with flame ionization detection (HS-GC-FID). Qualitative identification of fluorofentanyl (non-isomer specific) was undertaken by a protein precipitation extraction with acetonitrile followed by LC-QToF-MS detection. Limit of detection for fluorofentanyl was 0.5 ng/mL. Quantification was not completed. All other confirmatory analyses were completed by liquid chromatography with triple quadrupole mass spectrometry (LC-MS/MS) or gas chromatography with mass spectrometry (GC-MS).

Results: For the 11 month date range (06/01/2021 – 05/01/2022), we identified the presence of fluorofentanyl in 257 different postmortem blood samples across 9 different states: Arizona, Colorado, Indiana, Florida, Kansas, Michigan, Nebraska, North Dakota, and Ohio. Fluorofentanyl was most commonly detected alongside fentanyl (n=248), 4-ANPP (n=205), methamphetamine (n=86), acetylfentanyl (n=84), cocaine/benzoylecgonine (n=70), naloxone (n=65), Delta-9-THC/Delta-9-THC Carboxylic Acid (n=49), ethanol (n=47), gabapentin (n=31), and morphine (n=30). In the 248 cases that also had detectable fentanyl, the fentanyl blood concentrations ranged 0.1-476 ng/mL (mean, 30.4 ng/mL; standard deviation, 58.9 ng/mL). In the 257 cases, other NPS detected included metonitazene (n=26), flunitazene (n=4), isotonitazene (n=3), brorphine (n=3), flualprazolam (n=3), and N-pyrrolidinoetonitazene (n=2), and eutylone (n=1). Other prevalence data will be presented.

Conclusion/Discussion: Fluorofentanyl was detected in 257 cases in our laboratory from 06/01/2021 – 05/01/2022. It was most commonly detected alongside fentanyl, 4-ANPP, methamphetamine, acetylfentanyl, and cocaine/benzoylecgonine. It is prudent that a forensic toxicology laboratory assesses drug trends and prevalence for the locations which submit work to them and adapt their scopes of testing. If a laboratory is not screening for fluorofentanyl, it is possible to be missing potential positive casework.

P1 - Development of a solid phase extraction method for fentanyl analogs in biological matrices for analysis by LC-MS/ MS

Alli Timmons*, M.S., A-ABFT^{1,2}, Dr. Thomas Jourdan, Ph. D², Danielle Ross-Carr, M.S.¹. ¹Oklahoma State Bureau of Investigation – Forensic Science Center. ²University of Central Oklahoma – Forensic Science Institute, Edmond, Oklahoma.

Background/Introduction: To combat the looming Opioid Crisis, the federal government allotted funds to law enforcement laboratories to incorporate emerging opioids into their reporting capabilities. Fentanyl and its analogs (fentalogs) have proven to be one of the most significant threats amongst these compounds, with several having many hundreds to thousands of times the strength of morphine. Due to their immense potency, more and more sensitive methods are needed for their detection.

Objectives: The Oklahoma State Bureau of Investigation (OSBI) has invested in the development and validation of a robust solid-phase extraction (SPE) method for identification of fentalogs by liquid chromatography tandem mass spectrometry (LC-MS/MS). In addition to two isotopically-labeled internal standards, 14 compounds including the parent drug fentanyl were validated for qualitative identification in whole blood and urine matrices involving DUI/DUID and drug-facilitated crimes (DFCs).

Methods: The analytes in this method include: 4-ANPP, 4-FIBF/PFBF, acetyl fentanyl, acryl fentanyl, alpha-methyl fentanyl, butyryl fentanyl, cyclopropyl/crotonyl fentanyl, fentanyl, fluorofentanyl, furanyl fentanyl, methoxy acetyl fentanyl, norfentanyl, sufentanil, and valeryl fentanyl. Fentanyl – 13C6 and betahydroxythiofentanyl – 13C6 served as internal standards. The Biotage® application note on the analysis of fentalogs in whole blood was developed, expanded, and validated for use by the OSBI. It was adapted to use reagents available in our laboratory and incorporate identification in urine. 100 µL of sample was preconditioned with 0.1% formic acid spiked with the internal standards and loaded onto 30 mg/ mL 1- mL Biotage® EVOLUTE® EXPRESS CX SPE cartridges on a Thermo Scientific™ HyperSep™ Positive Pressure Manifold. They were washed with 1 mL of each of the following: deionized water, 0.1% formic acid, and methanol. The columns were dried for ~1 minute at ~20 psi and eluted with two 760 µL aliquots of a 39:10:1 ratio of ethyl acetate, acetonitrile, and concentrated ammonium hydroxide followed by evaporation under nitrogen. The sample was reconstituted with 50 µL of a 1:1 mixture of 0.1% formic acid in water (Mobile Phase A) and 0.1% formic acid in acetonitrile (Mobile Phase B). A Shimadzu 8050 LC-MS/MS with a Raptor™ Biphenyl HPLC column was used for analysis. SOFT 2022 Abstract Submission Form Due by June 10, 2021

Results: This method was validated following the successful completion of five studies: interference, carry over, ion suppression/enhancement (ISE), limit of detection (LOD), and stability. No quantitative studies were performed. The low positive control (LPC) concentrations were 0.1 ng/mL or 0.5 ng/mL based on literature recommendations. The high positive control concentration was 10x the LPC. No significant carry-over was seen at 28 ng/mL. In addition to 81 commonly encountered drugs, several isomers were assessed for interference. Reporting of indistinguishable compounds was modified to include all possibilities. For example, ortho- and para-Fluorofentanyl could not be chromatographically separated and therefore reported as "Flourofentanyl" without a prefix. LODs ranged from 0.05 – 0.5 ng/mL. Five compounds were above the recommended 25% suppression/enhancement. The impact of ISE on the LOD was evaluated and found to have no impact. A five-day stability study was performed on five sets of extracted, refrigerated samples. Chromatography remained consistent and reporting criteria were met for all results across the study.

Conclusion/Discussion: This method was successfully validated for the qualitative identification of 14 fentanyl-related compounds in whole blood and urine for DUI/DUID and DFCs committed in Oklahoma.

Reference: Biotage. (2019). Sample Preparation for Fentanyl Analogs in Whole-Blood. Retrieved June 5, 2019, from https://sampleprep.biotage.com/forensicdrugsofabuse.

P2 - ELISA detection of 7 PCP analogs in whole blood using Immunalysis phencyclidine (PCP) direct ELISA kit

Alyssa Austin^{*1}, Kimberly McCall² and Karen S Scott³. ¹Arcadia University, Glenside, PA. ²Immunalysis, Ponoma, CA. ³University of Alabama at Birmingham, Birmingham, AL.

Background/Introduction: Phencyclidine (PCP) is a dissociative drug that was developed as an anesthetic in the 1950s, however its use was discontinued due to its harmful effects on patients. It is now classified as a Schedule II substance. PCP analogs are substances that are chemically similar to PCP that create similar physiological effects on the body. As with other drugs PCP analogs have appeared on the market. NPS Discovery Trend Reports have listed 3-methoxy PCP, 3-hydroxy PCP, and CI-PCP as analogs that have been identified in biological samples in 2020, 2021 and 2022. Additionally, the SOFT NPS Committee recommended these analogs on the 2021 Recommended Scope for NPS Testing in the United States. Immunoassays are used as screening tests for these kinds of substances from biological matrices. Small modifications in the structure of drugs can invalidate these assays rendering them ineffective. For this reason, it is necessary to evaluate the cross reactivity's of new drugs as they become available on the NPS market.

Objectives: This experiment aimed to evaluate the cross-reactivity of 3-chloro PCP, 3-hydroxy PCP, 3 methoxy PCP, 4-methoxy PCP, 3-methyl PCP, and 4-hydroxymethyl PCP with PCP on an Immunalysis PCP Direct ELISA Kit.

Methods: Stock solutions with a concentration of 1 µg/mL in methanol were prepared for PCP and each analog. Concentrations of 2 ng/mL, 5 ng/mL, 10 ng/mL, and 20 ng/mL of PCP in bovine blood were prepared. The analogs were prepared at concentrations of 2 ng/mL, 5 ng/mL, 5 ng/mL, and 10 ng/mL. The analysis was performed according to the instructions provided in the Immunalysis kit. Each sample was analyzed in triplicate. A Tecan Sunrise microplate reader was used to measure the absorbance of the samples at 450 nm. The cross reactivity was evaluated by comparing the response of the analogs to the response of PCP.

Results: 3-Chloro PCP, 3-hydroxy PCP, 3-methoxy PCP, 4-methoxy PCP, 3-methyl PCP, 4-methyl PCP, and 4-hydroxymethyl PCP were determined to cross react on the Immunalysis PCP Direct ELISA Kit. Cross reactivity was determined to be 115%, 79.4%, 161%, 115%, 129%, 79.9%, and 95.1% for 3-chloro PCP, 3-hydroxy PCP, 3-methoxy PCP, 4-methoxy PCP, 3-methyl PCP, 4-methyl PCP, and 4-hydroxymethyl PCP, respectively.

Conclusion/Discussion: The data indicated that the Immunalysis PCP Direct ELISA Kit is effective at detecting these analogs of PCP.

P3 - Expanding NPS screening capabilities in the forensic toxicology laboratory

Casey Burrows^{*1} and Pierre Negri¹. ¹SCIEX, Redwood City, CA, USA.

Background/Introduction: The emergence and rising number of novel psychoactive substances (NPS) on the recreational drug market continues to pose health and safety challenges worldwide. These concerns are highlighting the need for timely screening approaches capable of accurately detecting and identifying these substances. The use of high-resolution instrumentation has provided a unique tool for screening unknown compounds such as NPS in complex biological samples with little or no method optimization. Here, we demonstrate a robust and comprehensive workflow on the SCIEX X500R QTOF system enabling users to screen for a list of over 900 compounds in biological matrices in a single injection method.

Objectives: The objective of this study was to develop a highly robust and comprehensive screening workflow that enhances the screening capabilities of NPS that have recently emerged on the recreational drug market.

Methods: A sub-panel of 900 compounds made up of 130 NPS and 9 internal standards were selected based on NPS trends and monitoring information. Analytes were extracted from urine using a dilute and shoot sample preparation procedure and from human whole blood using a protein precipitation procedure. Separations were performed on a Phenomenex Kinetex Phenyl-Hexyl column ($50 \times 4.6 \text{ mm}$, $2.6 \mu \text{m}$, 00B-4495-E0). Mobile phases used were ammonium formate in water and methanol with appropriate additives. The flow rate was 0.7 mL/min. The injection volume was 10 μ L and the total LC runtime was 9.5 minutes. MS and MS/MS data were collected using IDA and SWATH DIA on the SCIEX X500R QTOF system in positive mode. For IDA, a TOF MS/MS full scan ranging from 25 to 650 Da was acquired to ensure all fragments were captured for identification. For SWATH DIA, 14 variable Q1 windows ranging from 25 to 650 Da were acquired.

Results: The robustness of the developed method was investigated by analyzing the extracted human urine and whole blood samples at concentrations ranging from 0.5 to 100 ng/mL. Calibration curves were generated to each of the 130 NPS included in the panel demonstrated and showed excellent correlation, with R² values greater than 0.99 for all the NPS targeted in the panel, regardless of the acquisition method used. The assay showed great reproducibility with intra- and inter-day precision (%CV) values below 10% for all the calibrator solutions, proving the quantitative robustness of the two untargeted acquisition methods in both human urine and whole blood samples.

The ability of the method to accurately detect and identify NPS was investigated by analyzing discarded authentic postmortem case samples. Analyte identification was performed by reviewing the XIC, TOF MS and TOF MS/MS spectra with library match for each of the NPS included in the screening workflow. Analysis of one of the case samples showed the detection of the same 6 compounds with the addition Alpha-PPP, one of the stimulants included in this panel. A precursor mass error of -0.9 ppm, retention time error of 0.10% and MS/MS fit score of 98.2 provided excellent measures for the confident identification of this analyte in the case sample.

Conclusion/Discussion: A comprehensive and highly sensitive method for the screening and identification of over 900 NPS is described. The results demonstrated the quantification performance with excellent linearity, reproducibility and robust-ness. Compound identification was confirmed using MS/MS spectral library matching by leveraging the fragment-rich TOF MS/MS spectral information which enabled accurate compound identification of the 130 NPS using defined confidence criteria. Overall, the developed method provides the ability to screen for a list of over 900 compounds in complex biological specimens a single injection method.

P4 - Two-year prevalence of NPS in Québec (Canada): the success of a dynamic analytical method paired to an internal early warning system

Souryvanh Nirasay*, Catherine Amireault, Jaclin Ouimet, Béatrice Garneau, Brigitte Desharnais, Pascal Mireault. Laboratoire de sciences judiciaires et de médecine légale, Department of Toxicology, 1701 Parthenais St., Montréal, Québec, Canada, H2K 3S7.

Background/Introduction: Given the constant evolution of the novel psychoactive substances (NPS) market, systematically screening for these substances is a challenge for toxicology laboratories. In 2019, the *Laboratoire de sciences judiciaires et de médecine légale* (LSJML) initiated a comprehensive process to deal with this issue (Garneau *et al.*, Forensic Science International 318 (2021) 110595). An internal early warning system (EWS) identified new trends by monitoring detection events inside and outside the laboratory. This includes results from in-house non-targeted screening methods (GC-MS, QToF) as well as data from external partners (Health Canada (seized drugs), public health, drug checking services, new NPS alerts). An NPS of particular concern is rapidly added to the systematic screening method following a swift but complete validation procedure. All toxicology cases handled by the laboratory undergo NPS screening with this dynamic method.

Objectives: NPS prevalence in casework received between January 2020 and December 2021 was examined. Trends are compared to other jurisdictions, and efficiency of the comprehensive NPS process was evaluated.

Methods: As detailed in Garneau *et al.*, extraction of blood and urine samples was performed by a protein precipitation extraction. The diluted supernatant was analysed by LC-MS/MS using multiple reaction monitoring. This method was accredited under ISO 17025 and the SCC Requirements and Guidance (Forensics).

Since this was a dynamic method, the panel of analytes covered varied through time, as some NPS emerged and were added to the scope, while others were removed following extended periods of non-detection. Initially, 55 NPS and metabolites were screened; this number dropped to 49 after 3 years (March 2022). Flualprazolam (October 2019), isotonitazene (May 2020), 2-fluorodeschloroketamine, adinazolam, clonazolam, 8-aminoclonazolam, N-ethylheptedrone and protonitazene (September 2021) were added, while 12 opioid-type NPS and metabolites were removed.

Results: Between January 2020 and December 2021, one or more NPS was detected in 6% of all cases handled by the Department of Toxicology. These 943 cases were spread between driving under the influence of drugs (DUID, 67%), postmortem (30%) and sexual assaults (3%), a sharp contrast with the general caseload distribution (37% DUID, 51% postmortem, 12% sexual assaults).

Benzodiazepine-type NPS constituted 84% of findings, including etizolam (n=389), flubromazolam (n=305) and flualprazolam (n=279). EWS activities and analytical results indicated a rising prevalence of benzimidazole novel synthetic opioids (NSO). Whereas carfentanil was not detected in 2020, there were 24 occurrences in 2021. As for isotonitazene and protonitazene, 39 and 6 detection events occurred respectively in the 20 and 4 months following their introduction.

Conclusion/Discussion: NPS prevalence in the province of Québec followed a distinct pattern from other provinces or states. Whereas NSO were the main finding in several western provinces, these constituted only 9% of Québec's NPS detections. Furthermore, detection of synthetic cannabinoids in seized drugs or via the non-targeted screening remained almost non-existent, thus explaining their absence from the systematic screening panel. Benzodiazepines NPS were how-ever highly prevalent, so much so that etizolam, flualprazolam and flubromazolam are now part of the top 10 active drugs encountered in DUID cases alongside cocaine, methamphetamine and cannabis.

With a detection rate more than 6 times higher than it was prior to the implementation of the dynamic analytical method paired to an internal EWS (0.76%), this comprehensive process has proven an invaluable tool in tackling the NPS challenge.

P5 - Pharmacological characterization of new, generic ban evading synthetic cannabinoids

Marie H. Deventer¹, Katleen Van Uytfanghe¹, Inge M.J. Vinckier², Fabiano Reniero³, Claude Guillou³, **Christophe P. Stove^{*1}.** ¹Laboratory of Toxicology, Faculty of Pharmaceutical Sciences, Ghent University, Ghent, Belgium. ²Laboratory of Customs and Excises, Vilvoorde, Belgium. ³European Commission, Joint Research Centre, Directorate F-Health, Consumers and Reference Materials, Ispra, Italy.

Background/Introduction: Controlling newly emerging drugs, such as synthetic cannabinoid receptor agonists (SCRAs), remains a never-ending challenge. They are typically abused for their psychoactive effects, related to their cannabinoid 1 (CB₁) receptor activity. To evade control measures, in this case the 2021 Chinese class-wide SCRA ban, new compounds with previously unknown structural features are appearing on the recreational drug market, e.g. the recently detected ADB-FUBIATA and 5 "OXIZID" SCRAs. Both are not covered by this new ban, as ADB-FUBIATA carries an additional methylene group between the core and the linker and the OXIZIDS carry a previously unknown oxoindolin core. As is the case for most newly detected substances, their pharmacological properties are often unknown, leaving users unaware of the potential harms they may be exposed to.



Objectives: This study is the first to pharmacologically characterize these compounds at both cannabinoid receptors ($CB_1 \& CB_2$). Furthermore, 2 samples seized by the Belgian customs and confirmed to contain ADB-FUBIATA and BZO-4en-POXIZID, were analytically and pharmacologically characterized.

Methods: Potency and efficacy were assessed using live cell activity-based β arr2 recruitment assays, based on functional complementation of a split nanoluciferase. Concentrations from 25 (10) μ M to 0.01 nM were tested. The seized samples containing either ADB-FUBIATA or BZO-4en-POXIZID were characterized via liquid chromatography coupled to time-of-flight mass spectrometry, gas chromatography coupled to mass spectrometry, high-performance liquid chromatography, coupled to diode-array detection, Fourier transform infrared spectroscopy and nuclear magnetic resonance.

Results: All compounds moderately activated CB_1 , exhibiting a lower potency than common SCRAs JWH-018 and CP55,940. Focusing on the OXIZIDs, the n-hexyl OXIZID analog BZO-HEXOXIZID was the least potent and efficacious of the set. Shortening the tail structure to a pentyl tail (BZO-POXIZID and its fluorinated counterpart 5F-BZO-POXIZID) resulted in increased potency and efficacy at both CB_1 and CB_2 , however this increase was far less pronounced in case of an unsaturated pentenyl tail (BZO-4en-POXIZID). Overall, the cyclohexyl methyl analog BZO-CHMOXIZID had the highest potency and relative efficacy at both receptors. All OXIZID SCRAs were found to have a preference for CB_2 . On the other hand, ADB-FUBIATA had a moderate potency and efficacy at CB_1 . Interestingly, it failed to activate CB_2 , but showed antagonistic properties at 10 and 2.5 μ M. Based on the activity data, compared to the respective reference standard, a high purity of the seized samples could be assumed. Analytical characterization revealed that the ADB-FUBIATA powder contained a minor impurity, of which the exact chemical structure could not be fully identified. The presence of the fluorophenyl indole structure, also present in ADB-FUBIATA, was confirmed by NMR but the identity of the head group could not be determined. No impurities were detected in the BZO-4en-POXIZID powder. **Conclusion/Discussion:** This characterization of previously unknown SCRAs contributes to a broader insight into the properties of recently detected substances. It remains difficult to predict to what extent these new compounds will become popular on the recreational drug market, but it is expected that more SCRAs containing unexpected never-seen-before structural features will sooner or later make their appearance. The information provided here is relevant, as it informs about the potential harms of new substances, allowing risk prioritization and a more efficient legal response.

P6 - Quantitative analysis of novel psychoactive substances in various postmortem matrices using standard addition

Claire Kaspar*, Samantha Delor, Szabolcs Sofalvi, Christie Mitchell-Mata, Luigino Apollonio, Thomas Gilson. Cuyahoga County Medical Examiner's Office. Cleveland, Ohio.

Background/Introduction: In 2021, there were 671 fatal overdoses in Cuyahoga County, Ohio. Fentanyl and/or fentanyl analogues were detected in 490 (73%) of those cases. In addition to fentanyl/analogues, the Toxicology Unit at the Cuyahoga County Medical Examiner's Office (CCMEO) has seen a number of novel psychoactive substances (NPS) in casework. The most frequently detected NPS opioids at CCMEO over the past two years have been the "nitazenes" (2-benzylbenzimidazoles), isotonitazene and metonitazene. In 2021, etodesnitazene was detected in one postmortem case. Additionally, the NPS 2-Methyl-AP-237 (2-MAP-237) was detected in two postmortem cases, one each in 2020 and 2021.

In April of 2022, the State of Ohio Board of Pharmacy scheduled several nitazene drugs. As substances are scheduled, new drugs may emerge on the market to replace the controlled substances. It is important for toxicology laboratories to adapt to this changing drug market. This task can be challenging due to availability of certified reference materials (CRM) for NPS, the cost of CRMs, and the time required to revalidate existing procedures to include NPS. The method of standard addition (MSA) allows laboratories to provide a quantitative value for NPS analytes in a variety of matrices when full validation is not feasible.

Objectives: To quantitate isotonitazene, metonitazene, etodesnitazene and 2-MAP-237 in various postmortem specimens using the method of standard addition; to provide quantitative results for select postmortem cases where these NPS drugs were determined to contribute to cause of death; to offer information on postmortem redistribution for these analytes.

Methods: Cases from 2021 through the first quarter of 2022 were reviewed for the presence of isotonitazene, metonitazene, etodesnitazene and 2-MAP-237. Cases in which these NPS drugs were the sole or main contributor to cause of death were selected for further study. Specimens were extracted and analyzed using an in-house fentanyl and analogues procedure (LC-MS/MS). MSA was performed to obtain quantitative results, utilizing fentanyl-D₅ as internal standard for each analyte.

Results: The cases included in this study were positive for isotonitazene (n=3) and metonitazene (n=1). Isotonitazene and metonitazene were confirmed in femoral blood, heart blood and vitreous humor at concentrations <10 ng/mL. Etodesnitazene was qualitatively confirmed in one case, however, further study is needed to determine a suitable internal standard for the analyte. 2-MAP-237 was quantitated in one case, with 312 ng/mL confirmed in the femoral blood and 100 ng/mL in the vitreous humor.

Conclusion/Discussion: NPS opioids are commonly detected in casework at CCMEO, most frequently with other opioids or illicit drugs. This work provides a valuable reference of postmortem specimen concentrations that can be associated to a fatal drug overdose. Femoral blood, heart blood and vitreous humor are suitable samples for detecting and quantitating NPS opioids, and MSA allows laboratories to provide a reliable quantitative value for NPS analytes in a variety of matrices. This is of significant advantage when availability of standards or limited laboratory resources prohibit full validation.

P7 - Investigative study of synthetic benzodiazepine detection using the Immunalysis[®] benzodiazepine ELISA kit in correlation with triple quadrupole LC/MS/MS and QTOF-LC/MS

C. Alexia Lewis^{*1,2}, Dr. Jarrad Wagner², Dr. Robert Johnson¹, Leanne Hazard¹, Beryl Landry¹. ¹Tarrant County Medical Examiner's Office, Fort Worth, TX, USA. ²Oklahoma State University, Stillwater, OK, USA.

Background/Introduction: Novel or new psychoactive substances (NPS) like synthetic or designer benzodiazepines are hurting our communities. Benzodiazepines are central nervous system (CNS) depressant drugs that beneficially induce calmness, drowsiness, and sleep by modulating the effects of the inhibitory neurotransmitter GABA at different GABA receptors throughout the CNS. Designer benzodiazepines are a class of NPS that suppress the CNS like more common licit benzodiazepines. They are commonly mixed with opioids and stimulants, then pressed into illicit pharmaceutical pills. The Tarrant County Medical Examiner's Office utilizes an enzyme-linked immunosorbent assay (ELISA) screening method on Tecan's Freedom Evo instrument using Immunalysis[®] ELISA kits. Eight different assays are included in the panel, and one of those is benzodiazepines.

Objective: This presentation will discuss the preparation and screening of twenty-nine relevant synthetic benzodiazepines to investigate the utility of the Immunalysis[©] benzodiazepine ELISA kit for qualitative screening of each of the synthetic benzodiazepines tested and their possible response on a Shimadzu quadrupole time of flight liquid chromatography mass spectrometry (QTOF-LC/MS) and a Shimadzu liquid chromatography with tandem mass spectrometry (LC/ MS/MS).

Methods: For each synthetic benzodiazepine, two neat 10 mL solutions were prepared in methanol at 200 ng/mL and 1000 ng/mL for blood and urine, respectively. These stock solutions were prepared from a unique lot of methanolic standards obtained from the manufacturer. These stock solutions were subsequently used to prepare 3 blood samples and 3 urine samples for each of the synthetic benzodiazepines to be processed on the Tecan Freedom EVO 75 workstation. The 200 ng/mL methanolic neat solutions was used to prepare blood samples at concentrations of 10, 20, and 50 ng/mL and the 1000 ng/mL methanolic neat solution was used to prepare urine samples at concentrations of 50, 100, 250 ng/mL.

Results: Through investigation of these techniques, it has been shown that the ELISA screening method for benzodiazepines is reliable for most synthetic benzodiazepines. This presentation will provide the results of the ELISA study of synthetic benzodiazepine in blood and urine matrices at different concentrations, along with QTOF-LC/MS and LC/MS/MS instrumentation results.

Conclusion/Discussion: ELISA was proven to be a rapid and reproducible screening method for most synthetic benzodiazepines. Four synthetic benzodiazepines were not detected in blood at all concentrations tested. Meclonazepam, nifoxipam, bentazepam, and tofisopam showed no cross-reactivity to the Immunalysis[®] benzodiazepine kit during the blood ELISA run. Three synthetic benzodiazepines screened negative in urine at all concentrations tested. Nifoxipam, bentazepam, and tofisopam showed no cross-reactivity to the Immunalysis[®] benzodiazepine kit during the urine ELISA run. At low blood and urine concentrations, norflunitrazepam, nimetazepam, bromazepam, ketazolam, cinazepam, and etizolam did not always cross-react with the Immunalysis[®] benzodiazepine kit.

P8 - Leveraging sensitivity improvements for low-level detection of drugs, metabolites, and endogenous hormones in complex biological matrices

Crystal Holt*1, Pierre Negri1, Ian Moore2, Adrian Taylor2, Dan Blake3

¹SCIEX, Redwood City, CA, USA; ²SCIEX, Concord, Canada, SCIEX, Mereside, UK.

Background/Introduction: The ability to accurately quantify low levels of analytes in a wide variety of complex biological matrices is a critical requirement for many bioanalytical workflows. In the forensic laboratory, expanding the number of analytes per method—while maintaining the required level of laboratory throughput—is becoming increasingly challenging given the complexity and diversity of the relevant matrices in which the analytes need to be detected. Here, we demonstrate how the technological innovations on the SCIEX 7500 system are leveraged to improve the sensitivity and overall quantification of low-level analytes in multiple biological matrices.

Objectives: The objective of this study was to demonstrate how the sensitivity improvements afforded by the SCIEX 7500 are enabling forensic toxicologists to accurately detect and quantify low levels of drugs, metabolites, and endogenous hormones in a number of challenging forensic workflows.

Methods: Various panels of drugs including novel psychoactive substances (NPS, novel synthetic opioids (NSO), endogenous hormones and metabolites were used for these experiments. Separations were performed using a variety of Phenomenex columns and mobile phase compositions. Different LC methods were used for each panel. These compounds were extracted from a number of complex biological matrices including human whole blood, dried blood spots (DBS), oral fluid and plasma. Extraction procedures varied based on the biological matrix analyzed. A SCIEX 7500 system and a QTRAP 6500+ system were used for comparison. Both instruments were optimized for maximum sensitivity. Multiple reaction monitoring (MRM) was used to monitor the transitions for the suite of analytes targeted in the different methods.

Results: For each of the presented workflows, the normalized distribution of the peak areas (N=3) for each of the two MRM transitions monitored for the analytes was plotted, to compare the gain of the SCIEX 7500 system over the QTRAP 6500+ system. For a panel of 49 drugs and metabolites extracted from human whole blood samples, the peak area gains for the analytes ranged from 5.48 to 13.09-fold when using the SCIEX 7500 system. This averaged 8.72-fold increase in peak areas ultimately resulted in higher signals, greater S/N and lower limits of quantification (LLOQ) for all compounds, transitions, and replicates included in this workflow. For the analysis of 32 fentanyl analog isomers and novel synthetic opioids (NSO) extracted from human whole blood samples, sensitivity gains were significant on the SCIEX 7500 system, with average peak area increases of 8.37-fold for the 64 transitions monitored. For a panel of of 24 drugs and metabolites extracted from DBS, peak areas for the 24 analytes targeted in this assay were 7.25x higher, on average, when using the SCIEX 7500 system. For THC-COOH in oral fluid, the area gains were 7.9x (MRM) while the signal-to-noise gains were 3.1x (MRM). For the analysis of estrogens in human plasma, an average improvement in signal-to-noise of 4.7 was observed.

Conclusion/Discussion: The sensitivity improvements afforded by the SCIEX 7500 system to accurately detect and quantify low levels of drugs, metabolites, and endogenous hormones is described for a number of forensic workflows. The resulting increase in sensitivity can be leveraged to achieve robust and accurate quantification of previously undetectable compounds. These sensitivity improvements can also be leveraged to simplify sample preparation procedures, perform larger dilutions, and use lower sample and injection volumes, which have the added benefits of minimizing matrix effects and maintaining ionization efficiencies. Overall, the use of the SCIEX 7500 system drastically improves laboratory efficiency through potentially reduced complexity of sample preparation procedures, resulting in improved laboratory productivity and overall performance.

P9 - The method of standard addition with weighting factor as a tool for the reliable and efficient quantitation of postmortem toxicology and forensic casework

Danai Taruvinga*, Szabolcs Sofalvi. Toxicology Unit, Cuyahoga County Medical Examiner's Office (CCMEO), Cleveland, Ohio.

Background/Introduction: The continuous emergence of novel psychoactive substances (NPS) demands that forensic toxicology laboratories keep pace by developing and validating methods for the quantitation of some of these drugs. While method development for the quantitation of new drugs is in progress, laboratories are left with the option of either qualitatively reporting or identifying and funding reference laboratories for quantitative analysis. The method of standard addition (MSA) presents itself as an appropriate solution that allows for the efficient and reliable quantitation of NPS drugs.

Objective: Our objective is to explain the concept of MSA and identify and report the most accurate and dependable way it can be used as a tool for the quantitation of drugs that have yet to be validated on a traditional external calibration method. More specifically, we aim to explore the strengths and limitations of this method by comparing MSA with the inclusion of internal standardization, and by investigating the appropriateness of calibration curve weighting factors when using MSA in accordance with the Academy Standards Board Standard 054, First Edition's guidelines.

Method: Quantitation was achieved by using an unfortified case sample and an additional three concentration levels for each case. The accuracy of MSA was evaluated by fortifying sheep blood with 29 compounds that include Z-drugs, traditional benzodiazepines and biomarkers, and NPS benzodiazepines at low, medium, and high concentrations. Additionally, a selection of authentic human specimens previously reported qualitatively (8-aminoclonazolam, bromazolam) and an external proficiency were analyzed by the MSA protocol. The MSA protocol mirrored our lab's current validated benzodiazepine analysis method in that the drugs were extracted by solid-phase extraction, and the subsequent analysis was performed via LC-MS/MS. Comparisons of the resultant data that excluded internal standardization were made to that which included internal standardization and weighting factors of 1 and 1/x.

Results: Quantitations achieved using MSA with the inclusion of internal standardization and a 1/x weighting factor improved the analytical bias and accuracy of the assay. The fortified samples revealed that the accuracy of this method is largely dependent on the type of internal standard used, the separation of the points on the curve, and how low the sample's concentration is with respect to the three additional concentration levels. Notably, the external proficiency that contained zolpidem at a target concentration of 180 ng/mL was successfully quantified at 179 ng/mL following a five-fold dilution. The quantitation attempts of 8-aminoclonazolam and bromazolam revealed the importance of relying on the estimated concentration of the drug in question via a one-point curve to aid with the appropriate strategic preparation of the additional three concentration levels for each case.

Conclusion/Discussion: Given the evolving landscape of NPS drugs, the incorporation of MSA for quantitative reporting in forensic toxicology will likely become more prevalent. This work sought to optimize the application of MSA and demonstrated that when certain parameters such as the inclusion of internal standardization coupled with a weighting factor of 1/x are applied to MSA, the result is an overall improvement of the performance of the assay. The efficiency and reliability of the method materializes when the aforementioned parameters are applied to the assay, making it a quick and valuable tool for the quantitation of forensic casework.

P10 - Rapid and reliable quantification of six antipsychotic drugs in serum

David Brokaw^{*1}, Jing Lianyan² and Li Guoqing². ¹SCIEX, Redwood City, CA, USA. ²Shanghai, China.

Background/Introduction: Antipsychotics, also referred to as neuroleptics, a class of psychotropic drugs commonly prescribed to treat a wide range of psychotic disorders, work by altering the brain chemistry to help manage and relieve psychotic symptoms anxiety, serious agitation, violent or disruptive behavior and mania symptoms. Here we introduce a rapid and efficient extraction method in combination with the QTRAP 4500 system as a comprehensive and sensitive quantitative solution for high-throughput detection of six antipsychotic drugs in serum.

Objectives: The objective of this study was to develop a fast, reliable, and reproducible workflow for monitoring antipsychotic drug levels in biological matrix.

Methods: A total of six antipsychotic drugs (amisulpride, parlipidone, citalopram, risperidone, olanzapine, clozapine) were targeted in this workflow. Antipsychotic drugs were extracted from 50 μ L spiked serum samples using a protein precipitation procedure. Analytes were chromatographically separated using an XDB C18 LC column (50 × 4.6 mm, 1.8 μ m) held at 50°C. Mobile phase A (MPA) and mobile phase B (MPB) consisted of ammonium formate in water and ammonium formate in acetonitrile, respectively. The injection volume was 3 μ L and the total LC runtime was 3 minutes. MS and MS/MS data were acquired using positive electrospray ionization on the QTRAP 4500 system. The MRM algorithm was used to collect the appropriate amount of data points for quantifiable data. A single acquisition method consisting of 14 MRM (8 for the analytes and 6 for the internal standards) was created and used for analysis of the samples.

Results: A series of serum samples spiked with the six drugs were injected at six concentration levels ranging from 2 to 2000 ng/mL to assess the quantitative performance of the developed method. Excellent linearity was observed across the concentration ranges analyzed with R² values greater than 0.99 for each of the six analytes. The efficiency of the sample preparation procedure used in this study was investigated by calculating drug recoveries at two calibration levels. The sample preparation procedure used in this experiment demonstrated excellent recoveries with values ranging between 88.46% and 93.26%. The measurement reproducibility was also investigated at the same two concentration levels for each of the six analytes. The %RSD values ranged from 1.35% and 4.84%, demonstrating the high level of reproducibility of the method.

Conclusion/Discussion: A comprehensive workflow for the detection of six antipsychotic drugs in serum is described. The combination of an efficient sample preparation procedure and a fast and robust acquisition method enabled accurate detection of the antipsychotic drugs in serum samples. The optimized LC conditions resulted in separation of the drugs in a 3-minute runtime while the use of the MRM workflow enabled sensitive and accurate quantification of the six antipsychotic drugs across six calibration levels. The protein precipitation procedure for sample preparation demonstrated high recovery levels for the 6 antipsychotic drugs targeted in this workflow. In addition, the robustness of the developed workflow enabled reproducible and sensitive quantification of the six targeted antipsychotic drugs in serum, making this method compatible with the high-throughput requirements of modern forensic laboratories.

P11 - Investigation of the cross-reactivity of clonazolam, flualprazolam and flubromazolam with benzodiazepines lateral flow immunoassay tests

Matthew Rogers¹, Thomas Dore¹ and Eduardo G. de Campos^{*1}.

¹Department of Chemistry and Fermentation Sciences, Appalachian State University, Boone, NC 28608, USA. *

Background/Introduction: Designer benzodiazepines (DBZD) are novel psychoactive substances that share structural core similarity with traditional benzodiazepines (BZD). In the literature, clonazolam, flualprazolam and flubromazolam have been recently recommended to be included in the scope of routine testing in toxicology laboratories¹. Immunoassay test strips might also detect these compounds. However, information about the cross-reactivity of DBZD with BZD immunoassays is still limited and further investigation can be useful.

Objectives: To investigate the cross-reactivity of clonazolam, flualprazolam and flubromazolam with commercially available lateral flow immunochromatographic tests designed for the detection of BZD.

Methods: Reference materials were purchased from Cayman Chemical or Millipore-Sigma at 1 mg/mL solutions for alprazolam, clonazolam, flualprazolam, and flubromazolam. Innovative Research Inc Pooled Human Urine and Germaine[™] Laboratories AimScreen[™] Urine DipDevice for Benzodiazepines (with a cutoff of 300 ng/mL) were purchased from Fisher Scientific. Assays were performed at three different concentrations for each DBZD (low, 10 ng/mL; medium, 100 ng/mL; and high, 400 ng/mL), in triplicate. Negative (blank urine) and positive (alprazolam at 300 ng/mL) controls were analyzed in each batch. The procedure consisted in spiking 1.5 mL of blank urine with an aliquot of each standard solution (not greater than 5% of the total volume) and briefly homogenizing the sample. Immunoassays were performed by inserting the test strip in the urine sample for 15 s and the results were read in 3 to 5 minutes, according to the manufacturer instructions. Each test strip was analyzed by two independent analysts. The results were expressed similarly to the work of Bergh et al. (2021): "++"as clearly positive, "+" as positive, "(+)" as weak positive/ambiguous and "-" as negative².

Results: At 10 ng/mL, clonazolam, flualprazolam and flubromazolam did not exhibit significant cross-reactivity. However, increasing the concentration of the three DBZD to 100 and 400 ng/mL resulted in potential cross-reactivity with the immunoassay test. The medium concentration (100 ng/mL) resulted in challenging interpretation with the results being either considered positive or weak positive/ambiguous by the two analysts. No invalid tests were obtained during all experiments. All high concentration (400 ng/mL) samples of flualprazolam and flubromazolam were considered positive but not clearly positive as the positive control (alprazolam). For clonazolam, the readings for high concentration samples varied between positive, clearly positive and weak positive/ambiguous among the two analysts.

Conclusion/Discussion: Our results suggest that the DBZD at higher concentrations likely cross-react with lateral flow immunoassay tests designed for the detection of BZD, whereas the 10 ng/mL concentration did not yield toxicologically relevant results. Although some results were challenging to interpret, the use of immunoassay test strips can be a useful preliminary tool for screening the presence of DBZD in urine, which must be further confirmed by instrumental techniques. Our findings are also in line with other findings previously reported in the literature for flualprazolam and flubromazolam with a different type of immunoassay test³. However, concentrations of 10 ng/mL or less in urine have been reported for these DBZD, and the immunoassay might not be able to detect these low levels⁴.

References:

- 1. Center for Forensic Science Research and Education (CFSRE). NPS Scope Recommendations. https://www.npsdiscovery.org/reports/nps-scope-recommendations/ (2022).
- 2. Bergh, M. S.-S. et al. Int. J. Drug Policy 90, 103065 (2021).
- 3. Shapiro, A. et al. *Detection of etizolam, flualprazolam, and flubromazolam by benzodiazepine-specific lateral flow immunoassay test strips*. https://www.bccsu.ca/wp-content/uploads/2020/08/BenzoTestStrip_Report.pdf (2020).
- 4. Pettersson Bergstrand, M., et al. *Clin. Mass Spectrom.* **10**, 25–32 (2018).

P12 - A qualitative analysis of THC isomers and derivatives in e-liquids

Emani R. Smith, B.S.^{*1}, Kimberly N. Karin, M.S.², Justin L. Poklis, B.S.², Michelle R. Peace, Ph.D.¹ ¹Department of Forensic Science,² Department of Pharmacology & Toxicology, Virginia Commonwealth University, Richmond, VA.

Background/Introduction: E-cigarette liquids are a preferred modality for the consumption of synthesized THC isomers and derivatives. These cannabimimetics are labeled as "hemp-derived" to provide the market illusion that they are extracted from plant material as opposed to synthesized. These isomers include delta 8-tetrahydrocannabinol (Δ^{8} -THC), delta 6a,10a-tetrahydrocannabinol ($\Delta^{6a,10a}$ -THC), delta 10-tetrahydrocannabinol (Δ^{10} -THC), hexahydrocannabinol (HHC), tetrahydrocannabiphorol (THCP), and THC-O (THC acetate). Potency, efficacy, and the adverse effects are largely unknown and pose a public health risk.

Objectives: To analyze commercially available e-liquids for the presence of "hemp-derived" natural and unnatural cannabinoids using GC-MS and DART TOF-MS.

Methods: Twenty-six "hemp-derived" e-liquids were purchased from online retailers. The e-liquids were diluted in methanol and analyzed on a Jeol JMS T100LC Accu-TOF DART-MS and a Shimadzu QP2020 GC-MS. The DART TOF-MS was operated in positive mode with orifice 1 alternating between 20, 30, 60, and 90 V, while orifice 2 was operated at 5 V with a ring lens voltage of 3 V. The heater was set to 350°C with a helium flow rate for the ion source being 2.0 L/min. Voltages for the discharge needle, detector, and peaks voltage were set to 4000, 2000, and 800 V respectively. Calibration was achieved using polyethylene glycol 600 in methanol and a quality control containing cocaine, methamphetamine, and nefazodone was used to confirm masses fall within 5 mmu of the target along the m/z range. The mass range was 500 to 1,000 m/z. The e-liquids were analyzed using a Shimadzu QP-2020 with a HP-5MS column (30 m, 0.25 mm id, 0.25 μ m). The total run time was 26.33 mins with the temperature gradient starting at 70°C for 1 min, ramping 15°C/min until 300°C and held at 300°C for 10 mins. Mass spectra from the DART TOF-MS and GC-MS retention times and mass spectra of constituents in the e-liquids were compared to available analytical reference material. NIST, SWGDRUG, and Cayman Spectral libraries were used for the identification of THC isomers and derivatives when reference material was not available.

Results: The DART TOF-MS method was successful in identifying the "hemp-derived" THC isomers and derivatives but was unsuccessful in resolving isobaric compounds. The GC-MS method was successful in separating and identifying the "hemp-derived" THC isomers and associated isobars. In the 26 purchased e-liquids, a variety of cannabinoids including Δ^8 , Δ^{10} -THC, THC-O, THCP, or HHC were identified. The products were determined to contain mixtures of these analytes, as well as phytocannabinoids. All products contained the THC isomers and derivatives, with the exception of products labeled to contain Δ^{10} -THC. Of the 26 products, 22 contained additional THC isomers or derivatives that were not labeled. As an example, "God's Gift", was labeled to contain THCP, but the acetate forms of Δ^8 Δ^9 -THC, and CBD were also identified. Another product labeled to contain THC-O, "Mowie Wowie", was determined to contain the acetates for each of the following cannabinoids identified: Δ^8 Δ^9 , Δ^{10} -THC, cannabinol (CBN), and CBD. An HHC product, "Lucid Blue", was determined to contain HHC, $\Delta^{6a,10a}$ -THC, and CBN.

Conclusion/Discussion: HHC, Δ^8 , Δ^9 , $\Delta^{6a,10a}$ -THC, THCP, and THC-O in addition to cannabidiol-di-acetate (CBD-di-O) and other cannabinoid acetate derivatives were identified in the commercially available products using DART TOF-MS and GC-MS. These THC isomers and derivatives are synthesized from CBD are the new wave of emerging cannabinoids and cannabimimetics sold legally as hemp-derived products in the market. Currently, limited information regarding the potency, efficacy, and potential toxidrome of these compounds is known, putting public health and safety at risk.

P13 - Case report: identification of AP-238 and 2-fluorodeschloroketamine in internet available powder samples

Karissa N. Resnik, M.S.¹, Justin Poklis, B.S.², Michelle Peace, Ph.D.¹, F. Gerard Moeller, M.D.³, **Emanuele A. Alves, Ph.D.***^{1,4} ¹Department of Forensic Science, Virginia Commonwealth University, Richmond, VA, 23284. ²Department of Pharmacology and Toxicology, Virginia Commonwealth University, Richmond, VA, 23284. ³Department of Psychiatry, Virginia Commonwealth University, Richmond, VA, 23284. ⁴National School of Public Health Sergio Arouca, Oswaldo Cruz Foundation, Rio de Janeiro, Brazil.

Background/Introduction: The rate of opioid use increased drastically in the United States, and the country has experienced an enormous increase in deaths from opioid overdose, opioid use disorder (OUD), and other harms. With the advent of the cryptocurrency and the easy access of substances on the Darknet, new synthetic opioids (NSO) became broadly available in the US, which presents a public health threat. NSO are substances considered opioid agonists, and usually present high potency when compared to morphine. The risk associated to the consumption of unknown samples purchased from the internet relies on the lack of identification of the real content of these samples. Among the NSOs currently identified in the U.S., the piperazine derivatives such as bucinnazine, MT-45, AD-1211, and 2-methyl-AP-237, has brought attention. 2-fluoro-deschloroketamine (2F-DCK) is a NPS that has been identified in approximately 20 cases between January and July 2019. In most of these cases, 2F-DCK was identified alongside other ketamine-type drugs. AP-238 is a structural isomer of 2-methyl-AP-237 and an analog of bucinnazine. It belongs to the cinnamylpiperazine subclass and is the fourth analog in this series of compounds to be reported by NPS Discovery. At present, most NSOs are not scheduled in the U.S., as they are not a therapeutic choice for the treatment of pain. Powdered samples suspected to contain bucinnazine were obtained and analyzed using DART-MS and a non-targeted GC-MS analysis approach.

Objectives: The aim of this study was to identify the compounds present in two powder samples purchased on the internet suspected to contain bucinnazine.

Methods: The two powder samples were dissolved in methanol to a concentration of approximately 1 mg/mL, and then were diluted in methanol to a concentration of 20 μ g/mL. The samples were then screened on a Jeol JMS-T100LC ion-Sense DART with an AccuTOF MS (Jeol, Tokyo, Japan). The samples were wanded in front of the sample injection port 5 times each. The temperature was 300°C and the DART-TOF-MS was operated in positive mode. Samples were additionally screened on a Shimadzu GC-MS QP-2020 instrument (Shimadzu Corporation, Kyoto, Japan). The column used was an Agilent HP-5MS 30 m x 0.250 mm x 0.250 μ m column (Agilent, Santa Clara, CA). The injection temperature was 250°C, the column oven temperature was 70°C for 1 minute and then was ramped to 300°C over 15 minutes, then held for 10.0 minutes. The total run time of the method was 26.33 minutes. The ion source temperature was 250°C. The GC was in splitless mode, and the MS was in SCAN mode with a range of 40.00 *m/z* to 550.00 *m/z*.

Results: The screening of the two unknown powders indicated that neither sample contained bucinnazine. The first unknown powder was found to contain 2-fluorodeschloroketamine as its primary constituent, and the second unknown powder was found to contain AP-238 as its primary constituent. The quantification of both powders showed that powder 1 presented 78.0% of 2-fluorodeschloroketamine, and powder 2 presented 88.9% of AP-238.

Conclusion/Discussion: Neither of the obtained powders contained bucinnazine, though both powders were advertised by the suppliers as containing bucinnazine. 2-fluoro-deschloroketamine (2F-DCK) and AP-238 were found to be present in the samples. Little data is available on the health risks of AP-237 or 2F-DCK. They are not currently scheduled in the U.S. but are advertised as bucinnazine. This data also adds to the large body of evidence that substances sold as one compound frequently contain other compounds not on the label, which are potentially riskier. The presence of psychoactive substances that are not labeled on the product or not properly described to be present are a public health concern and a significant risk to the consumer.

P14 - Solid phase extraction of novel synthetic 2-benzylbenzimidazole opioid compounds, "Nitazenes"

Emily Eng*, Stephanie Reichardt, Abderrahim Abdelkaoui, Ritesh Pandya, UCT, Bristol, PA.

Background/Introduction: First synthesized in the 1950s by a Swiss pharmaceutical company, etonitazene and its structurally related analogs were investigated as potential analgesics. However, these 2-benzylbenzimidazole "nitazene" compounds were never approved for clinical use. Today, they are re-appearing as new potent non-fentanyl-like synthetic opioids. Isotonitazene, also known as "iso" or "toni", first appeared in Canada and Europe in March 2019. In July of 2019, these compounds were first detected in biological samples in the US. In August 2020, the US Drug Enforcement Administration (DEA) temporarily placed Isotonitazene in Schedule I. In December 2021, the DEA issued temporary scheduling of seven additional nitazene compounds. Nicknamed "Frankenstein" opioids due to their highly potent nature, these compounds have been identified in numerous states and the number of cases continues to rise. This poster outlines methods for extracting nine nitazene compounds from urine and blood utilizing a polymeric cation exchange solid phase extraction (SPE) sorbent.

Objectives: To develop LC-MS/MS and SPE extraction methods for nitazene compounds in urine and blood that yield high recoveries with low matrix effects.

Methods: Nine nitazene compounds (butonitazene, clonitazene, etonitazene, flunitazene, isotonitazene, metodesnitazene, metonitazene, N-pyrrolidino etonitazene 'etonitazepyne' and pronitazene) were extracted from human urine and blood utilizing UCT's Styre Screen[®] BCX (SSBCX) SPE cartridges. 1 mL of biological sample was prepared by adding 1.7 mL of phosphate buffer (pH 7.0, 0.1M) followed by 300 μ L of acetonitrile for sample dilution and pH adjustment. The SSBCX SPE cartridges were conditioned with 3 mL of methanol and equilibrated with 3 mL of pH 7.0 phosphate buffer. Matrix interferences were removed by washing the columns with two washes of 3 mL of deionized water and two washes of 3 mL of 50% methanol. The target analytes were eluted with 3 mL of 2% ammonium hydroxide in methanol. After fully drying under a gentle stream of nitrogen at 30°C, the extracts were reconstituted in 1 mL of 50:50 methanol:water. Samples were analyzed using a Shimadzu Nexera LC-30AD with MS-8050 operated in positive ion mode. Analytes were separated using a SelectraCore[®] UHPLC C18 Column (100 mm x 2.1 mm, 2.7 µm) with 0.1% formic acid in water as mobile phase A and 0.1% formic acid in methanol as mobile phase B.

Results: Analytes were successfully extracted from urine and blood with high recoveries and low matrix effects. Recoveries were determined by comparing pre-spiked samples to post-spiked samples. The matrix effect was determined by comparing post-spiked samples to spiked solvent samples. Extraction recoveries of analytes from urine ranged from 95-99% with an average of 96% and the matrix effect for all analytes was within ± 25%. The relative standard deviation (RSD) for the urine extraction ranged from 6-15%. Extraction recoveries of analytes from blood ranged between 87-100% with an average of 95% and the matrix effect for all analytes was within ± 25%. The RSD values for the blood extraction ranged from 1-4%.

Conclusion/Discussion: Due to their novelty, much information was gained about this class of drugs and how they behave under specific conditions during method development. For example, a sizeable amount of some of the non-polar analytes, such as butonitazene and isotonitazene, remain in the test tube after loading the sample onto the SPE cartridge. Also, some analytes were significantly lost during evaporation of the elute under certain conditions. Potential solutions were tested to combat these issues. An extraction method has been successfully optimized to extract nine nitazene compounds from urine and blood using Styre Screen[®] BCX SPE cartridges.

P15 - A user's history of the mixed bag; designer opiates present in urine samples from heroin users, 2016-2022

Melissa Goggin; Gregory C. Janis*. Laboratory Corporation of America, Saint Paul, MN.

Background/Introduction: In 2016 we began sequestering a subset of clinical urine samples submitted for drug testing. Urine samples which screened positive for 6-acetylmorphine were de-identified and subjected to further testing looking for the presence of fentanyl, fentanyl analogs, and other designer opioids or related compounds. Designer opiates identified in samples from this ongoing project are compiled quarterly. Historic trends within the heroin using population are shown, defining the rise and fall of fentanyl and specific designer opiates.

Objectives: The laboratory surveyed urine samples for designer opiates with the goal of steering test offerings. The historical data provides insights into the timelines of individual designer opioids and intentional or unintentional polydrug use in the heroin using population.

Methods: Clinical urine samples ordered for comprehensive drug testing were subjected to a battery of screening procedures including a test to detect heroin use via the Thermo Scientific CEDIA® Heroin Metabolite (6-AM) Assay. Samples which screened positive on the heroin metabolite assay were de-identified and reanalyzed using a combination of procedures designed to detect designer opioids.

Subsequent testing targeted the following parent drugs and metabolites. The detection of metabolites was utilized as confirmational evidence supporting the detection of a parent drug. Not all compounds were targeted over the entire timeframe; additional drugs of concern were added to the panel as they became relevant and available.

Fentanyl	Acetylfentanyl	Carfentanil
Valeryl-fentanyl	Acetylfentanyl-4-methylphenethyl	3-Methylfentanyl
Furanyl-fentanyl	Butyryl-fentanyl	IC-26
para-Fluorofentanyl	β-hydroxy-thiofentanyl	Mitragynine
Ocfentanil	para-fluorobutyryl fentanyl	MT-45
AH-7921	4-Methoxy-butyrylfentanyl	U-47700
AH-8529	AH-8533	THF-fentanyl
Methoxy-Acetylfentanyl	Cyclopropyl-fentanyl	Fluoro-cyclopropyl fentanyl
Phenacetylfentanyl	Acrylfentanyl	Isotonitazene
Metonitazene	Xylazine	

Testing consisted of an initial 90 second LC-MS/MS screening procedure targeting a single MRM transition for each compound. A secondary conditional LC-MS/MS confirmation procedure was additionally developed and validated utilizing independent chromatographic parameters, multiple transitions per analyte, and additional targeted metabolites and internal standards. The lower limit of quantitation for the targeted drugs ranged from 250 to 1000 pg/mL.

Results: Since 2016, 3420 urine samples were selected for further testing based on positive screening results for 6-AM. At the start, just under 40% of those samples contained fentanyl or norfentanyl, and less than 20% of the samples contained any other identifiable designer opioids. The presence of fentanyl then climbed to over 80% in 2018 and has since declined to levels below those of 2016. Because this study only examines samples which screened positive for 6-AM, it likely under-represents the overall positivity rate for fentanyl (i.e. a sample from a fentanyl-only user would not meet the inclusion criteria). Though the prevalence of fentanyl in these samples has shifted over time, fentanyl has remained the most prevalent finding. Acetylfentanyl has been a second consistent finding over the study period; its prevalence has

ranged from approximately 7%, climbed to above 35%, and then returned to below 10%. Furanyl fentanyl and fluorobutyryl fentanyl both had periods of being the second most dominant finding (above acetyfentanyl), peaking at over 35% and near 20% respectively. However, both of these compounds dropped to being a minor finding after approximately a two year window. The only other fentanyl analog to be found present in more than 10% of samples was para-fluorofentanyl at the end of 2021. In 2022 metonitazene, isonitazine, and xylazine were added to the list of targets. While metonitazine and isonitazine remain rare findings, xylazine was found in approximately 10% of samples screening positive for 6-AM.

Conclusion/Discussion: This study highlights the shifting landscape of polydrug use combining heroin with designer opioids. By far fentanyl is the dominant synthetic opioid consumed by heroin users in the US. No other designer opioids appeared more prevalent or more consistently over the period between 2016 and 2022.

P16 - Quantitative analysis of 10 anticoagulants in human blood by UPLC triple quadrupole mass spectrometry (LCMSMS)

Hiu Yu Lam^{*1}, Tais R. Fiorentin¹, Francis X. Diamond¹, Amanda L.A. Mohr¹, Barry K. Logan^{1,2} ¹The Center for Forensic Science Research & Education, Willow Grove, PA.

²NMS Labs, Horsham, PA.

Background/Introduction: Anticoagulant agents within the warfarin and superwarfarin class are commonly used in commercial rodenticides. Currently, these drugs are unregulated and commercially available for purchase. As such, they have been implicated in forensic casework including suicides, homicides, and accidental poisonings. Toxic clinical effects of anticoagulant exposure include spontaneous internal and external bleeding, which can result in death. Recently, anticoagulant drugs have emerged as toxic adulterants within the illicit drug supply. Two major anticoagulant outbreaks, Chicago (2018) and Tampa (2021) have been reported wherein synthetic cannabinoids were laced with anticoagulant drugs, which led to at least five deaths and numerous hospitalizations. The detection and quantification of these compounds in forensic casework is challenging due to the low concentrations of the drugs, and the fact that they are typically not detected in routine screening workflows.

Objectives: The objective of this project was to develop and validate a quantitative, targeted method to isolate ten anticoagulants from human blood with subsequent analysis by liquid chromatography coupled with triple quadrupole mass spectrometry (LCMSMS).

Methods: Sample extracts were analyzed using a Water ACQUITY I-class UPLC coupled with a Waters Xevo TQ-S micro tandem mass spectrometer. A reverse phase gradient was performed using 0.02% ammonium hydroxide in water (MPA) and 0.02% ammonium hydroxide in methanol (MPB). Analytical separation was performed using a Water BEH C₁₈ column (1.7 μ m, 2.1 x 100 mm) with a total run time of four minutes. The mass spectrometer operated in negative-ion electrospray ionization mode for ten anticoagulant compounds: brodifacoum, bromadiolone, chlorophacinone, coumatetralyl, difenacoum, difethialone, diphacinone, flocoumafen, pindone, and warfarin. The analytes and internal standards were monitored and analyzed in multiple reaction monitoring (MRM) mode, with cone voltages and collision energies varied among analytes.

Results: This method was validated in accordance with standards set by Academy Standards Broads in Forensic Toxicology (ANSI/ASB Standard 036). Parameters of method validation included calibration, bias/precision, limit of quantification (LOQ), limit of detection (LOD), carryover, interferences, and ionization suppression/enhancement. Additional parameters evaluated included recovery, auto-sampler stability, and matrix matching. The calibration model was constructed with seven-point calibrators ranging 5 to 250 ng/mL with quality control set at three different concentrations: 15, 100 and 200 ng/mL. The LOD was administratively set at 1 ng/mL. All parameters of validation were met.

Following validation, authentic de-identified samples (n=79) from the recent outbreak in Florida were tested. In 74 samples, brodifacoum was detected and quantitated across three different matrices (blood, serum, and plasma). In six serum samples, difenacoum was detected with three of those samples being quantitated.

Drug	Matrix	Mean concentra- tion (ng/mL)	Median (ng/mL)	Range (ng/mL)
Brodifacoum	Blood(n=13)	159.5(±131)	110	48.8-429.8
	Serum(n=45)	575.9(±432)	476.3	86.2-1995
	Plasma(n=16)	134.3(±133)	53.2	11.5-365.3
Difenacoum	Serum(n=6)	7.4(±2.2)*	7.4*	5.3-9.6*

*Quantified in three samples only.

Conclusion/Discussion: The method was highly efficient for the extraction of anticoagulant drugs, which are highly lipophilic. The analytical method proved to be sufficiently sensitive and specific for the detection and quantitation of ten anticoagulant drugs in human blood or other matrices. With respect to authentic cases, brodifacoum was identified in authentic cases in three different matrices. From a clinical perspective, the findings from this research demonstrate the utility 107

of detection anticoagulant drugs in serum using the described extraction technique and method. Additionally, some serum samples were also positive for difenacoum, which was detected at much lower concentrations relative to brodifacoum. Laboratories should consider including additional anticoagulant drugs into their panels. As the number of incidents involving anticoagulant drugs adulterating seized material increases, laboratories should be aware of the challenges associated with the detection of anticoagulant drugs and consider incorporating these drugs into their panels.
P17 - The unseen impact of $\Delta 8$ -THC products on the regulated drug testing industry

Jarod Kabulski*, David Kuntz, Sarah Small, Joseph McCrary, Kyle Bockenstedt, Steve Huffman, Richard Hewitt, Melissa Beals. Clinical Reference Laboratory. Lenexa, Kansas.

Background/Introduction: In 2020, an unknown and unresolved chromatographic peak appearing in the Δ 9-Carboxy-THC confirmation assay resulted in an increase in the reporting of specimens as invalid due to LCMS/MS interference. The escalating occurrence of this undetermined interference initiated an investigation into the isolation and identification of the compound, as its retention time, parent ion, and product ions were the same as those for Δ 9-Carboxy-THC. Through extensive chromatographic analysis and the re-validation of the Δ 9-Carboxy-THC LC-MS/MS confirmation assay to provide compound resolution, it was possible to elucidate the existence of a once rarely seen metabolite, Δ 8-Carboxy-THC, in regulated workplace specimens.

Objectives: Determine the prevalence of regulated specimens containing $\Delta 8$ -THC using retrospective analysis of chromatography.

Methods: Specimens were analyzed by LC-MS/MS to separate, identify, and quantitate Δ 9-Carboxy-THC from Δ 8-Carboxy-THC for DOT specimens. Federally regulated confirmation batches were manually examined to assess the peak presence of Δ 8-Carboxy-THC with Δ 9-Carboxy-THC. The chromatographic review window allowed for observation of both Δ 8Carboxy-THC and Δ 9-Carboxy-THC with baseline separation. For reporting purposes, only Δ 9Carboxy-THC was evaluated for quantitation and peak acceptance based on NLCP criteria. THC immunoassay results were compared to corresponding Δ 9Carboxy-THC LC-MS/MS results to determine screen and confirmation positivity rates.

Results: LC-MS/MS confirmation data was evaluated from April 2020 to May 2022. Although the immunoassay reagent in use remained constant throughout this period, the rate of screened positive samples (50 ng/mL cutoff) to confirmed positive samples (15 ng/mL cutoff) demonstrated a steady decline, just as availability of Δ 8-THC products in stores and online was growing. In April of 2020, the Δ 9Carboxy-THC confirmation rate was 96%; that number had decreased to 78% by May of 2022. Further analysis incorporated creatinine levels in order to take state of hydration into consideration; however, data did not support excessive hydration as the cause of the lower confirmation rate. Correlation of results across the normal creatinine range showed that samples analyzed typically contained around 4 ng/mL of Δ 9Carboxy-THC. Only seven positive-screening samples did not contain Δ 8 or Δ 9-Carboxy-THC, most likely exhibiting immunoassay cross-reactivity due to Protonix or other unidentified cannabinoids, such as Δ 10-Carboxy-THC.

Conclusion/Discussion:

Data trends indicate the increasing prevalence of Δ 8-Carboxy-THC positive specimens going unreported. The federal drug testing program specifically identifies Δ 9-Carboxy-THC as the reportable metabolite for marijuana use, and Δ 8-THC products are specifically promoted as the "safe alternative" that is legal and/or can't be detected. Concentrations of Δ 8-Carboxy-THC in the urine are often remarkably high, frequently into the hundreds of ng/mL and greater. With the emergence of easily obtainable Δ 8-THC products, employer drug testing programs should consider the inclusion of Δ 8-Carboxy-THC in addition to Δ 9Carboxy-THC, as more than 20% of immunoassay results are negative with this omission.

P18 - Development of an extraction method for the analysis of synthetic opioids in bone samples using the bead ruptor

Kala N. Babb, B.S.^{*1}, David J. Cohen, M.D.², Justin L. Poklis, B.S.³, Emanuele A. Alves, Ph.D.^{1,4}. ¹Department of Forensic Science, Virginia Commonwealth University, Richmond VA, 23284; ²Department of Biomedical Engineering, Virginia Commonwealth University, Richmond VA, 23284; ³Department of Pharmacology & Toxicology, Virginia Commonwealth University, Richmond VA, 23284; ³Department of Public Health Sergio Arouca, Oswaldo Cruz Foundation, Rio de Janeiro, RJ, 21040-360, Brazil.

Background/Introduction: Synthetic opioids have been the leading cause of overdose deaths compared to other opioid types in recent years. Many of these deaths are attributed to novel synthetic opioids (NSO). Detecting NSOs in toxicological matrices can prove difficult due to their high potencies leading to low concentrations in the body and a general lack of literature on these substances. In the case of extreme decomposition or significant scavenging, the only toxicological matrix that may be available is bone tissue. In these instances, establishing blood:bone drug concentration correlation is important to help determine the cause of death. To achieve this, correlation studies of animal models are necessary, and a careful choice of a suitable animal model is required. Rabbits, as opposed to rodents, are the most appropriate choice, due to their exhibition of spontaneous cortical bone remodeling that is not present in rodents. Previous research has not yet been able to establish such a correlation using rodents, leading to studies with a new choice of animal model for appropriate correlation data.

Objectives: The aim of this work is to develop a fast and suitable method for the extraction of synthetic opioids from bone samples. This method will be applied to establish blood:bone drug correlation using rabbits as the chosen animal model. The method homogenizes bone samples and extracts the analyte simultaneously utilizing the Omni International Bead Ruptor Elite. A method to detect and quantify NSOs on the GC-MS was also established.

Methods: For the development of the extraction protocol, commercial drug-free rabbit bone samples were fortified with the following synthetic opioid standards: fentanyl, isovaleryl fentanyl, tetrahydrofuran fentanyl, bucinnazine, 2-methyl-AP-237, and AP-238. Bones were soaked for 12 hours in a buffer solution containing the analytes. Two sets (#1 and #2) of 500 mg fortified bones were added to a 15 mL tube with 4 metal beads containing 4 mL of 4:1 butyl acetate:ethyl ether and 0.5 mL ammonium hydroxide (extraction #1), and 4 mL 3% ammonium hydroxide in 20:80 isopropanol:ethyl acetate (extraction #2) for the homogenization process with the Omni International Bead Ruptor Elite. The supernatants were collected from both extractions. Extraction #1 underwent additional wash steps and then submitted for GC-MS analysis. Extraction #2 was evaporated and reconstituted with 50 µL ethyl acetate for GC-MS analysis. A quant GC-MS method was developed to identify and quantitate the selected NSOs, and calibration curves were prepared using the standard addition method.

Results: Both extraction procedures presented promising recoveries. Yields for all analytes varied from 33.63% to 76.20% for extraction #1, and from 32.05% to 68.79% for extraction #2. The use of the Bead Ruptor for the extraction during the homogenization process showed to be efficient with less time and cost spent. The GC-MS method showed sufficient linearity for all analytes at a linear range of 10-5000 ng/mL. The lowest R² equaled 0.9984 for tetrahydrofuran fentanyl and the highest R² equaled 0.9999 for fentanyl. The validation of this method is ongoing.

Conclusion/Discussion: The developed extraction and GC-MS methods were capable of extracting, detecting, and quantitating the target opioids. Further optimization in extraction solvents would be required, as well as comparing between LLE and SPE to determine which gives the highest yield as well as highest data quality. Once animal experiments begin, these methods can be applied to the bone samples obtained from the rabbits for the determination of the blood:bone correlation of synthetic opioids. P19 -Highly sensitive MS/MS detection for confident identification of potent novel synthetic opioids (NSO) and their metabolites

Karl Oetjen^{1*}, Pierre Negri¹ and Alex J. Krotulski². ¹SCIEX, Redwood City, CA, USA; ²Center for Forensic Science Research and Education at the Fredric Rieders Family Foundation, USA.

Background/Introduction: The continuous emergence of NSO on the recreational drug market is creating an additional challenge for drug tracking agencies and laboratories to meet. In recent years, high-resolution mass spectrometry has provided forensic toxicology laboratories with a unique tool for the untargeted detection and identification of these new emerging substances, with little or no method optimization necessary. As newer and more potent synthetic opioids are synthetized and introduced to the recreational drug market, timely and sensitive analytical drug screening approaches focused on untargeted drug identification these new substances are critically needed. Here we introduce a highly sensitive method, using the new SCIEX ZenoTOF 7600 system, for the detection and identification of potent NSO compounds in human whole blood.

Objectives: The objective of this study was to develop a highly sensitive drug screening workflow for the detection and identification of low levels NSO, metabolites and other potent drugs in discarded authentic postmortem case samples.

Methods: A panel of NSO consisting of 3 newly emerging non-fentanyl opioids (brorphine, isotonitazene, metonitazene), one metabolite (4'-hydroxy nitazene) and two halogenated fentanyl analogs (para-fluorofentanyl and para-chlorofentanyl) was selected for method development. NSO were extracted from 500 μ L of spiked whole blood mixtures using a liquid-liquid extraction (LLE) procedure. Analytes were chromatographically separated using a Phenomenex Kinetex C18 column (50 × 3.0 mm, 2.6 μ m, 00B-4462-YO). Mobile phase A (MPA) and mobile phase B (MPB) were ammonium formate with formic acid and formic acid in methanol and acetonitrile, respectively. The injection volume was 10 μ L and the total LC runtime was 15.5 minutes. MS and MS/MS data were collected for each sample using Zeno IDA for optimal sensitivity on the ZenoTOF 7600 system. Data acquisition consisted of a TOF MS scan to collect accurate mass precursor ions from 100 to 700 Da, followed by a TOF MS/MS full scan ranging from 25 to 700 Da to ensure all fragments were captured for identification using a maximum of 16 candidate ions.

Results: A series of 9 calibrator solutions extracted from control human whole blood were injected to evaluate the quantitative performance of the system and its ability to accurately measure low level analytes with a high level of precision and accuracy in TOF MS mode. LLOQs of between 10 and 50pg/ml were observed for all compounds analyzed. Accuracies at LLOQ ranged from 86 – 111% and imprecision was <15% for all compounds analyzed.

The MS/MS sensitivity improvements resulting from the use of the Zeno trap on the ZenoTOF 7600 system was investigated by analyzing discarded authentic postmortem case samples from subjects suspected of NSO ingestion resulting in accidental overdoses. Results from the analysis of these discarded authentic postmortem case samples showed successful detection of low level NSO and metabolites at concentrations that were not previously achievable. The use of specific technologies afforded by the new SCIEX ZenoTOF 7600 system generated an average 9x sensitivity improvement on spectral data providing significantly improved library matching.

Conclusion/Discussion: A comprehensive and highly sensitive method for the screening and identification of potent NSO in human whole blood is described. The significant gains in MS/MS sensitivity on the SCIEX ZenoTOF 7600 system yielded an improvement in confident identifications of low-level analytes through spectral library matching. The observed sensitivity gains afforded by the use of the Zeno trap resulted in a 9x improvement, on average, in TOF MS/MS sensitivity across the drugs positively identified in the authentic case samples analyzed. The MS/MS sensitivity levels afforded by ZenoTOF 7600 system provide a means to monitor low levels of ultra-potent NSO in poly-drug intake scenarios.

P20 - Conversion of cannabidiol to tetrahydrocannabinol in acidic foods and beverages

Lawrance Mullen¹, E Dale Hart ¹, Ruth E. Winecker¹, Svante Vikingsson¹, Ronald Flegel³, Eugene Hayes³, Mahmoud ElSohly², Waseem Gul², Tim Murphy², Iram Shahzadi², Kareem ElSohly². ¹RTI International, Research Triangle Park, NC, 27709, USA; ²ElSohly Laboratories, Incorporated, Oxford, MS 38655, USA; ³Substance Abuse and Mental Health Services Administration, Rockville, MD, 20857 USA.

Background/Introduction: As cannabidiol (CBD) is now found in a growing number of foods and beverages, the potential of CBD to convert to tetrahydrocannabinol (THC) in acidic beverages or foods has become a concern. In a previously conducted study of commercially available food and beverages containing CBD with pH <7 presented at SOFT 2021, a number were found to have significant amounts of THC. As THC can be present in CBD source material, an experiment was designed using a THC-free CBD source material to see if THC would be produced in situ.

Objectives: To determine if CBD would convert to $\Delta 9$ -THC or any other cannabinoid (e.g., $\Delta 8$ -THC) when fortified into commercial beverages or food condiment products that have a pH of ≤ 4 at room temperature over a three-month period.

Methods: Three commercial beverages (cola drink, lemonade, and sports drink) and three condiments (hot sauce, ketchup, and mustard) purchased from a local grocery store were fortified with a nano-emulsion of CBD (THC-free) purchased from a local herbal supplement retail shop. Purity and concentration of the CBD was verified by reference testing. Target amounts in mg of CBD were at 1x and 4x typical amounts found in commercially available CBD-containing edible products.

The fortified products were divided into 20-mL aliquots and stored at room temperature for a period of three-months. Aliquots were submitted for quantitative cannabinoids analysis (CBD, $\Delta 9$ -THC, and $\Delta 8$ -THC) just after preparation, at 30-days and at 90-days and were tested by Liquid Chromatography Tandem Mass Spectrometry (LC-MS/MS) and Gas Chromatography coupled with a Flame Ionization Detector (FID) analytical methodology.

Results: Some conversion of CBD to Δ 9-THC was detected in all six products. **\Delta8-THC was not** detected in any product. The most significant conversion of CBD occurred in the lemonade beverage. For the two fortified lemonades, Lemonade #1 (20 mg CBD per 12 ounces) and Lemonade #2 (80 mg CBD per 12 ounces), Δ 9-THC was detected at all three time points (Table 1). At 90 days, Lemonade #2 contained 1.25 mg THC per 12 ounces. Cola beverage, which has a similar pH to lemonade, produced much less THC.

Table 1. CBD Conversion in CBD-spiked Lemonade and Cola								
Sample	CBD Added (µg/mL)	THC Initial*	THC 30 Days	THC 90 Days				
Lemonade #1 (20 mg CBD)	56.3	0.51 mg	0.14 mg	0.41 mg				
Lemonade #2 (80 mg CBD)	225.4	0.72 mg	0.86 mg	1.25 mg				
Cola #1 (20 mg CBD)	56.3	ND	0.05 mg	0.10 mg				
Cola #2 (80 mg CBD)	225.4	ND	0.06 mg	0.09 mg				
* THC per 12-ounce (355 mL)								

ND - none detected

Discussion/Conclusion: Conversion of CBD to THC can occur in some acidic food products when those products are stored at room temperature. Therefore, despite purchasing beverages manufactured with pure CBD, consumers might be at some risk of unknowingly ingesting small amounts of THC. The most significant conversion occurred with a lemonade beverage. Although lemonade and cola have similar pH's (~2.5), the same degree of CBD conversion with cola was not observed. This might indicate that citric acid in citrus beverages is more effective in promoting CBD conversion than the phosphoric acid in cola. The 30-day aliquot of the CBD in lemonade #1 result was noticeably lower than both the initial (time zero) and the 90-day aliquot. The same phenomenon was not observed with lemonade #2. Further experiments are underway to explain this result and to fully characterize the effect of different acids and beverages on CBD conversion to THC.

P21 - In silico and in vitro phase I biotransformation analysis of synthetic cathinones: eutylone and MPHP

Leonardo B. Maya, M.S.,* Anthony P. DeCaprio, Ph.D. Florida International University, Miami, Florida.

Background/Introduction: New psychoactive substances (NPS) refer to a category of newly modified or synthesized drugs designed to mimic pharmacological effects associated with classical illicit substances. Synthetic cathinones are stimulatory NPS synthesized to mimic the effects of cathinone compounds found in the *Catha edulis* shrub. Derivatives of these naturally occurring monoamine alkaloids are all similar in their core scaffolding, which contain a ketone at the β-position of the amino alkyl chain attached to the phenyl ring. The myriad of possible changes to the core structure results in varying physiological and psychological effects and, most importantly for toxicological analyses, a wide array of metabolic products. This presents a challenge for identification and detection of parent drug and metabolic products. *In vitro* biotransformation studies of NPS, including synthetic cathinones, using human liver microsomes (HLM) are critical to identifying metabolites to be targeted for toxicologic analysis. This can be complemented and extended by *in silico* computational analysis of NPS biotransformation.

Objectives: The objective of this research was to provide an in-depth analysis of the phase I metabolism of two synthetic cathinones: eutylone and 4'-methyl- α -pyrrolidinohexiophenone (MPHP). Utilizing in silico methods to produce a biotransformation library for each NPS, this was comparatively assessed with in vitro HLM assays. Mass spectrometric data were analyzed to elucidate the identity of metabolites produced.

Methods: The NPS were selected based on class similarity and structural variety. Each NPS was subjected to phase 1 metabolism via *in silico* analysis using *MetaSite* metabolism prediction software. Each substrate had biotransformation predicted using a liver enzyme model. Each metabolite produced in this step was followed by another round of analysis to produce a second generation of predicted metabolites. For *in vitro* HLM biotransformation, a 25 mM buffer solution of pH 7.4 and 1 mg/mL of each drug was used. Cofactors of 1 mM NADPH, 1.5 mM glucose-6-phosphate, and 1.5 mM magnesium chloride, along with drug in buffer, were added to 0.5 mg/mL HLM and 0.2 U/mL glucose-6-phosphate dehydrogenase in a final volume of 250 uL, with a final drug concentration of 2 mM. The reaction mixture was incubated for 4 h at 37°C with constant mixing of 120 rpm, then centrifuged at 15,000 x g for 30 min. A 200 uL aliquot was analyzed on an Agilent 1290/6530 LC-QToF-MS system for identification of metabolites.

Results: Utilizing MetaSite *in silico* analysis, eutylone produced 21 primary metabolites of various biotransformations. Hydroxylation and dealkylation were the most common biotransformations, and 17 unique secondary metabolites were also produced from dealkylation, carbonylation, or dehydrogenation. MPHP produced 18 primary metabolites, with the majority being hydroxylated metabolites. In addition, a total of 16 secondary metabolites were produced from a subsequent carbonylation or hydroxylation. The HLM assays identified 13 total metabolites of eutylone (dehydrogenated amine metabolite; m/z 234, is represented in the mass spectrum below) and 10 total metabolites of MPHP that corresponded with the results produced via the *in silico* method.



Conclusion: The method herein described has proven to produce metabolites consistent with literature findings for eutylone. This method has also produced new information for MPHP. In addition, novel phase I metabolites with elaboration of fragmentation patterns for both drugs, not previously discovered, is detailed in this work. These results indicate that both *in silico* and *in vitro* biotransformation studies can improve the prediction of metabolites of NPS that can represent additional relevant targets for forensic toxicological screening.

P22 - Using untargeted high-resolution mass spectrometry and molecular networking for rapid identification of novel psychoactive substances

Maia Bates, BS*1, Heather Barkholtz, PhD2

¹University of Wisconsin – Madison, Department of Chemistry, Madison, WI

²University of Wisconsin – Madison, Department of Pharmaceutical Sciences, Madison, WI.

Background/Introduction: Drug abuse remains one of the biggest issues that our law enforcement agencies face today, and the increasing popularity of designer drugs makes detection and identification a real challenge. Novel psychoactive substances (NPS) are generated faster than available analytical detection methods and there are often no standard reference materials.

Objective: The objective of this research is to develop an untargeted data acquisition and molecular networking data processing approach for rapid identification of NPSs and metabolites in biological matrices.

Methods: Whole blood was spiked with 44 National Safety Council's (NSC) Tier I and II drugs of abuse (29 Tier I and 15 Tier II) at their suggested minimum reporting limit and high concentrations for analysis. For each substance studied, the desired concentrations were determined using the SOFT NPS Committee guidelines. The analytes were extracted from 100 µl of whole blood using a protein precipitation procedure and eluted through a 96-well Captiva EMR-Lipid extraction plate into collection wells. The analytes were then dried down and reconstituted in HPLC-grade methanol and centrifuged before loading onto the instrument for chromatographic separation. The separation was performed on a Waters Acquity HSS C₁₈ column (2.1 mm x 150 mm, 1.8 µm particles). Mobile phase A consisted of 5 mM ammonium formate at pH 3.0 and Mobile phase B consists of acetonitrile and 0.001% formic acid, these mobile phases were applied as a gradient at 50°C for 15 minutes in positive ion mode and 7.5 minutes in negative ion mode for each sample. Results from extracted samples were compared to neat solutions to identify analyte recovery and ionization suppression/ enhancement. MS and MS/MS data was collected using a Xevo QToF mass spectrometer and annotated using Waters UNIFI 3D peak algorithm. This data was then converted to open source mzML file format and used to populate a spectral library within the Global Natural Product Social Molecular Networking (GNPS) online MS/MS data analysis infrastructure.

Results: The compounds analyzed included: amphetamines, analgesics, benzodiazepines, cannabinoids, cathinones, fentanyl analogues, opioids, and stimulants. All were detected at the low and high recommended concentrations and the limit of detection (LOD) for these compounds were below the NSC's minimum reporting limits. The compounds were extracted and analyzed with small specimen volume and minimal solvent using the method outlined above. The analyte's recovery, ionization suppression/enhancement, LOD, and carryover were considered in the identification. Additionally, open-source MS/MS spectra of each drug considered in this work was exported from UNIFI and added to the GNPS platform in a public drugs of abuse spectral library.

Conclusion/Discussion: Untargeted time-of-flight mass spectrometry coupled with molecular networking can provide a framework for the rapid identification of NPS. To make this a reality, the untargeted data acquisition method must be validated. Additionally, a publicly available drugs of abuse spectral library must be curated within GNPS to be used to "seed" molecular networks of untargeted MS/MS spectra from casework.

Funding: This work was supported by the National Institute of Justice (NIJ) Research and Development in Forensic Science for Criminal Justice Purposes grant 15PNIJ-21-GG-04171-COAP. The opinions, findings, and conclusions or recommendations expressed in this publication are those of the author(s) and do not necessarily reflect those of the Department of Justice

P23 - Validation of novel synthetic cannabinoid ADB-BUTINACA with LC/MS/MS

Mary Susan Eley*, Erin Shonsey, Tara Federico, Rebekah Boswell, Curt Harper, Alabama Department of Forensic Sciences (ADFS), Hoover, Alabama.

Background/Introduction: ADB-BUTINACA [1-butyl-N-(1-carbamoyl-2,2-dimethyl-propyl)indazole-3-carboxamide] is a synthetic cannabinoid that was first identified in China in 2015. Over the past two years, it has emerged among seized drug and toxicological evidence in the United States. A notable rise in detection was reported by The Center for Forensic Research and Education in 2021. ADB-BUTINACA has also been detected in seized drug evidence at the Alabama Department of Forensic Sciences (ADFS).

Objectives: The objective was to validate ADB-BUTINACA qualitatively by LC/MS/MS.

Methods: Limit of detection (LOD), interference, carryover, and robustness studies were conducted in accordance to American National Standards Institute/Academy Standards Board (ANSI/ASB) guidelines. ADB-BUTINACA LOD studies were conducted over three days in duplicate at concentrations of 0.005, 0.01, 0.025, 0.05, and 0.1 ng/mL. A total of twelve negative matrices were analyzed for interferences (bovine, antemortem, and postmortem blood). Over 100 common analytes, including 26 synthetic cannabinoids, were also evaluated for interferences. Carryover was evaluated at three concentrations (10, 15, and 20 ng/mL). Blood samples spiked with ADB-BUTINACA were combined with a deuterated internal standard [JWH-250 N-(4-hydroxypentyl) metabolite-d₅]. A protein precipitation was used, and samples were analyzed on an Agilent 1260 Infinity Binary Liquid Chromatograph/Agilent 6460 Triple Quadrupole Mass Spectrometer (LC/MS/MS). The Agilent LC/MS/MS was equipped with a Poroshell 120 EC-C18 column (2.1 x 100 mm, 2.7 μ m) and mobile phases, (A) 5 mM Ammonium Formate with 0.1% Formic Acid and (B) Methanol with 0.1% Formic Acid. A 10-minute gradient run was used with dynamic multiple reaction monitoring (MRM).

Results: Identification criteria was set according to current ADFS synthetic cannabinoids operating procedures. This includes acceptable retention time, qualifier ratio, signal to noise, and chromatography. The LOD established at 0.025 ng/mL. No interference was detected with negative bovine, antemortem, and postmortem blood matrices, and no interference was detected with over 100 common analytes (e.g. meth/amphetamine class, opioids, cannabinoids, fentanyl/fentanyl analogues, benzodiazepines, etc.). Interference was also evaluated for 26 synthetic cannabinoids with only AB-PINACA (structural isomer of ADB-BUTINACA) containing the same qualifier transition. However, AB-PINACA did not interfere with the target ADB-BUTINACA qualifier transition. No carryover was noted with ADB-BUTINACA after injections containing 10 ng/mL, 15 ng/mL, and 20 ng/mL. Two postmortem case studies were identified and highlighted.

Conclusion/Discussion: To address the growing trend, the ADFS toxicology section validated a method for qualitatively identifying the synthetic cannabinoid, ADB-BUTINACA. This is an important addition to the current ADFS synthetic cannabinoids scope. Through this validation, ADB-BUTINACA was identified in two cases that were negative with our previous scope of analysis. Laboratories should consider adding this synthetic cannabinoid to their testing panel due to its emerging prevalence.

P24 - Methamphetamine and fentanyl use trends in Ohio and Michigan from 2017 to 2021

Matthew Levitas*, Matthew Borr, Bridget Lemberg, David Lemberg. Forensic Fluids Laboratories, Kalamazoo, Michigan.

Background/Introduction: Psychostimulant and synthetic opioid use has increased in the United States. Greater production and distribution of these drugs from Mexico are inundating multiple states and cities across the country. Moreover, deaths involving these drugs is increasing. In 2017, psychostimulants and synthetic opioids were involved in 7,826 and 20,932 deaths respectively. In 2021, they were involved in 25,129 and 58,875 deaths respectively. Methamphetamine and fentanyl are key perpetrators in overdose deaths. However, ex situ analyses of methamphetamine and fentanyl drug use in live user populations are limited, creating a need to explore their prevalence in these populations. Forensic Fluids Laboratories, an oral fluid toxicology laboratory, routinely tests for and confirms the presence of methamphetamine and fentanyl. As a laboratory that tests living user populations, Forensic Fluids sought to examine usage trends for these two drugs in Ohio and Michigan. Three user populations were examined: child protective services, community corrections, and probation courts programs.

Objectives: Our objective was to investigate methamphetamine and fentanyl positivity rate trends in Ohio and Michigan from 2017 through 2021. We examined these trends in the data at the aggregated state level, and at the county level to identify where methamphetamine and fentanyl positivity rates have increased or decreased.

Methods: Nationally collected statistics on psychostimulant and synthetic opioid use were obtained from the Centers for Disease Control using the Drug Overdose Surveillance and Epidemiology (DOSE) and National Vital Statistics System (NVSS) Provisional Drug Overdose Death Counts databases. The trends within these database systems were compared with confirmed toxicology results for methamphetamine and fentanyl collected by Forensic Fluids Laboratories, using Microsoft Excel.

Results: From 2017 to 2021, deaths in the United States involving psychostimulants and synthetic opioids increased 221% and 146% respectively. In this same five-year span, methamphetamine and fentanyl use increased in Ohio and Michigan. In Ohio, methamphetamine use by individuals tested for child protective services and probation court programs increased from 6.1% to 11.5%. Trends at the county levels showed 51 out of 75 counties tested had increased use in 2021 compared to 2017. Fentanyl use in the state increased from 2.4% to 4.8%. Trends at the county level showed 33 out of 75 counties tested had increased use in 2021 compared to 2017.

In Michigan, methamphetamine use by individuals tested for child protective services and probation court programs increased from 3.9% to 6.1%. Trends at the county level showed 17 out of 78 counties tested had increased use in 2021 compared to 2017. Fentanyl use in the state went from 38.5% in 2017 to 2.0% in 2019. Use of the drug was 2.4% in 2021.

Conclusion/Discussion:As the involvement of these two drug classes has increased in overdose involved deaths, so has the use of methamphetamine and fentanyl within individuals tested by child protective services, community corrections, and probation court programs in Ohio and Michigan. In Ohio, increases were observed year over year for both drugs. Data from Michigan was nuanced because of limited testing for the drugs during this time frame, especially 2020 and 2021. Only 23 counties in the state had methamphetamine testing data collected for 2020 and 2021, limiting the ability to discern trends. Additionally, fentanyl testing was inconsistent across years. In 2019, 41,453 fentanyl tests were performed across 78 counties. In 2020 however, only 1,976 fentanyl tests were performed across 18 counties. This variability in Michigan from year to year demonstrates a need for more robust testing moving forward.

P25 - Identification and quantitation of traditional and designer benzodiazepines in urine by UHPLC-MS/MS

Michael Clark*, Martin Jacques, Zayne Williams, Melissa Beals, David Kuntz; Clinical Reference Laboratory, Lenexa, KS.

Background/Introduction: Cannabinoid use has increased significantly subsequent to the ratification of the 2018 United States Farm Bill. A rugged analytical method for the identification and quantitation of cannabinoids is valuable in determining recreational and medical use of these compounds, as well as monitoring potential contaminations in over-the-counter cannabinoid products. The method developed by our laboratory allows for the determination of 15 different cannabinoids in urine.

Objectives: Develop an analytical method for the extraction, detection, and quantitation of (-)- Δ 9-THC, Δ 9CarboxyTHC, 11-Hydroxy- Δ 9-THC, Δ 9-Tetrahydrocannabivarin, Δ 9Carboxy-Tetrahydrocannabivarin, (-)- Δ 8-THC, Δ 8-Carboxy-THC, Cannabidiol, 7Hydroxy-Cannabidiol, 7-Carboxy-Cannabidiol, Cannabidiolic Acid, Cannabinol, Cannabichromene, Cannabigerol, and Cannabicyclol in urine by LC-MS/MS for a controlled dosing research study. This method was validated in accordance with NLCP Mass Spectrometry Guidelines.

Methods: Normal human urine fortified with bovine serum albumin was spiked with 15 cannabinoids at known concentrations and analyzed to establish linearity and evaluate assay interference and matrix effects. Sample preparation involved dual hydrolysis of a 0.500 mL aliquot of urine specimen using BG Turbo β -glucuronidase/0.1M phosphate buffer (pH 6.8) solution followed by the addition of 5N Potassium Hydroxide. Samples were neutralized with 5N Formic Acid and the mixture was eluted through an Agilent Captiva EMR—Lipid 3 mL Cartridge in a silanized glass tube. The cartridge was then rinsed with 80:20 Acetonitrile: DI H₂O and eluted into the same tube. A liquid-liquid extraction was performed using the eluent, pH 4.8 0.4M Ammonium Acetate buffer, and 2:1 Hexanes: Ethyl Acetate. The organic components were decanted, dried, and then reconstituted with 0.1% Formic Acid in 50:50 DI H₂O: Methanol. Analysis was executed by a Shimadzu Nexera LC40D X3 UHPLC equipped with a WatersTM CORTECS C18+ column coupled to a Sciex API7500 tandem mass spectrometer. The aqueous mobile phase (A), 0.1% Acetic Acid in water, and organic mobile phase (B), 0.1% Acetic Acid in Acetonitrile, flowed at a consistent rate of 0.5 mL/minute over the 15-minute run time. MS-MS analysis was conducted using electrospray ionization in both positive and negative MRM modes.

HPLC Gradient						
Time (min)	Pump A%	Pump B%				
0.50	53.0	47.0				
1.50	52.0	48.0				
2.50	44.0	56.0				
4.50	44.0	56.0				
5.50	37.5	62.5				
9.50	27.5	72.5				
12.00	27.5	72.5				
12.50	15.0	85.0				
13.50	15.0	85.0				
13.51	53.0	47.0				
14.51	53.0	47.0				
15.00	STOP					

Negative Ionization									
Analyte		Transitions	РТ	LOD/					
	Precursor	Quantifier	Qualifier	(mins)	LOQ (ng/ mL)	(ng/mL)			
7-COOH-CBD	343.2	179.0	231.1	3.51	0.50	100.0			
Δ9-COOH-THCV	315.2	217.1	163.1	3.78	0.50	100.0			
Δ8-COOH-THC	343.1	245.1	191.2	5.90	0.50	100.0			
Δ9-СООН-ТНС	343.1	245.2	191.1	6.13	0.50	100.0			
CBDA	357.2	245.1	227.1	9.02	0.50	100.0			
7-OH-CBD	329.2	268.1	179.0	3.72	0.50	100.0			
CBG	315.0	136.0	177.2	8.84	0.50	50.0			
Positive Ionization	Positive Ionization								
11-OH-THC	331.2	193.1	201.1	5.83	0.50	100.0			
THCV	287.3	135.0	123.0	8.53	1.00	100.0			
CBD	315.2	193.2	135.2	8.86	0.50	100.0			
CBN	311.1	241.0	208.0	10.40	0.50	100.0			
Δ9-THC	315.1	193.2	123.0	11.38	0.50	100.0			
Δ8-THC	315.1	193.2	135.3	11.65	0.50	50.0			
CBL	315.2	193.0	123.0	12.36	2.00	100.0			
CBC	315.2	193.1	123.1	12.91	2.00	100.0			

Results: Linearity was established for all analytes and no carryover was observed at the highest concentrations. Interference and matrix effect studies did not reveal any issues with identification, quantitation, or ion suppression.

Conclusion/Discussion: The analytical method reliably identified and quantitated 15 cannabinoids in urine at pg/mL levels, contributing to the scientific knowledge of cannabinoid metabolism and distribution in urine. This method demonstrated selectivity, accuracy, and reproducibility for federally-sponsored research studies.

P26 -Enhancing high-resolution mass spectrometry performance for NPS analysis with improved sensitivity and characterization

Pierre Negri^{1*} and Alex J. Krotulski². 1SCIEX, Redwood City, CA, USA; ²Center for Forensic Science Research and Education at the Fredric Rieders Family Foundation, USA.

Background/Introduction: The recent outburst of novel synthetic opioids (NSO) into the recreational drug market has been a major contributor to the ongoing opioid crisis. These substances are known to greatly vary in purity and potency, increasing risks of intoxications and accidental fatalities. Additionally, many classes of NPS such as newly emerging fentanyl opioids, novel synthetic opioids (NSO) and fentanyl analogs share similar structure and composition, adding another layer of complexity for forensic toxicologists tasked with their characterization and identification. As the emergence of structurally related NPS become more prevalent to evade regulations, the requirements for analytical characterization of these substances are consequently increasing

Objectives:The objective of this study was to use a newly developed fragmentation technique called electron activated dissociation (EAD) for the in-depth characterization and identification of challenging NPS.

Methods: A panel of 42 NPS including newly emerging fentanyl opioids, halogenated fentanyl analogs, synthetic opioids and synthetic cannabinoids was selected for method development. NPS were extracted from 500 μ L of spiked whole blood mixtures using a liquid-liquid extraction (LLE) procedure. Analytes were chromatographically separated using a Phenomenex Kinetex C18 column (50 × 3.0 mm, 2.6 μ m, 00B-4462-Y0). Mobile phase A (MPA) and mobile phase B (MPB) were ammonium formate with formic acid and formic acid in methanol and acetonitrile, respectively. The injection volume was 10 μ L and the total LC runtime was 15.5 minutes. MS and MS/MS data were collected for each sample using Zeno IDA on the ZenoTOF 7600 system using EAD as the fragmentation mechanism. Data acquisition consisted of a TOF MS scan to collect accurate mass precursor ions from 100 to 700 Da, followed by a TOF MS/MS full scan ranging from 25 to 700 Da to ensure all fragments were captured for identification.

Results: Neat standard solutions of the 42 targeted NPS were injected to optimize the EAD parameters and achieve optimal sensitivity, reproducibility, and selectivity of the generated fragment ions. The results demonstrated the ability of EAD to generate richer fragmentation by generating unique diagnostic fragment ions that enable in-depth characterization of these challenging NPS in discarded postmortem case sample. For example, the EAD-based TOF MS/MS spectra of two AP series isomers that were previously indistinguishable using the commonly used collision induced dissociation (CID) fragmentation mechanism showed unique fragments and spectral differences that enable differentiation of the three isobaric synthetic opioids. In the case of challenging synthetic cannabinoids, the EAD-based TOF MS/MS spectra generated unique spectral features that provide complementary structural information, enabling in-depth characterization synthetic cannabinoids. Finally, the presence of two unique fragment in the EAD-based TOF MS/MS spectrum of ortho-chlorofentanyl allowed its differentiation from its para- and meta-chlorofentanyl analogs, providing unambiguous evidence for the identification of this isomer in a discarded postmortem case sample, which was not achievable using CID. In addition, the use of the Zeno trap on the ZenoTOF 7600 system in combination with EAD resulted in the acquisition of much richer MS/MS spectra that contained unique, high-intensity fragment ions that can used for confident compound identification.

Conclusion/Discussion: The ability of EAD as an alternative fragmentation mechanism to generate unique, diagnostic fragment ions for the in-depth characterization and identification of challenging NPS is described. The results show that the robustness and reproducibility of EAD provide forensic toxicologists with a unique tool for the characterization, identification and differentiation of structurally similar and isobaric NPS. Overall, the technological enhancements of the ZenoTOF 7600 provide a high degree of sensitivity, selectivity and confidence for MS/MS-based characterization of NPS.

P27 - The rise and fall of nitazene analogs: using standard addition and a uniform analytical approach to rapidly respond to changes in trends

Rebecca Mastrovito, M.S.^{*(1)}, Donna Papsun, M.S., D-ABFT-FT ⁽¹⁾, Alex Krotulski, PhD ⁽²⁾, Barry Logan, PhD, F-ABFT ⁽¹⁾⁽²⁾. ⁽¹⁾ NMS Labs, Horsham, PA. ⁽²⁾ Center for Forensic Science Research and Education, Willow Grove, PA.

Background/Introduction: Nitazenes, formally known as 2-benzylbenzimidazole opioids, were originally synthesized in the 1950s in an effort to develop safer opioid analgesics. Isotonitazene, one of the prototypical compounds of this subclass, later emerged in authentic toxicology case work collected in 2019. The nitazenes gained popularity following a DEA control notice in 2018 which scheduled substances structurally related to fentanyl, with different compounds peaking at different points. Change in popularity within the nitazene subclass has been typically seen in response to individual drug scheduling, however, a broader control measure was issued in December 2021. The changes in popularity for isotonita-zene, metonitazene, N-pyrrolidino etonitazene, and protonitazene will be discussed.

Objectives: The objective of this presentation is to the discuss the emergence of a series of novel 2-benzylmidazole opioids between 2019 and 2022 with focus on temporal and geographical trends and quantitative results from postmortem casework.

Methods: Due to the emergence of this subclass of nitazenes, a uniform approach was developed for their detection. A single extraction protocol was developed and validated under an abbreviated workflow designed in consultation with the ANSI/ASB guidelines. Compounds were analyzed under the same instrument conditions while using the suspected analyte for purposes of standard addition.

Toxicological data reported between January 2020 and April 2022 were reviewed for nitazene findings. The presence of isotonitazene, metonitazene, and N-pyrrolidino etonitazene were presumptively identified using sample-mining with a surveillance library applied to screening by Liquid Chromatography Time-of-Flight Mass Spectrometry (LC-TOF/MS). Confirmatory testing for isotonitazene/protonitazene, metonitazene, and N-pyrrolidino etonitazene was achieved by standard addition on a UPLC system coupled to a tandem-quadrupole mass spectrometer (LC-MS/MS). Results: In total, 406 blood samples were submitted and confirmed for the presence of one or more specified nitazene. The first cases for isotonitazene were reported in January 2020, however, the oldest collection dates were from April 2019. A total of 162 cases were reported for isotonitazene between January 2020 and April 2022, with peak positivity in March 2020. Following the scheduling of isotonitazene in June 2020, metonitazene emerged in replacement. Metonitazene has been reported in 186 cases between April 2021 and April 2022, with peak positivity in August 2021. The nitazene subclass further diversified with the detection of N-pyrrolidino etonitazene and protonitazene by December 2021. N-Pyrrolidino etonitazene has been reported in 36 cases and protonitazene in 9 cases by April 2022. Of note, confirmation of protonitazene requires chromatographic separation due to being an isomer of isotonitazene. A heatmap with geographic distribution information will be included in subsequent presentation.

Conclusion/Discussion: Nitazenes are continuing to appear and proliferate in the illicit drug market. This analytical approach allowed for the rapid response for analytical confirmation as new compounds emerged over the past 2-3 years. The rapid emergence and short life spans are prohibitive for routine development and validation. This trend data further reiterates the need for continued vigilance in surveillance and detection for identification of novel synthetic opioids.

P28 - Comparing performance of LC-QqQ-MS vs. LC-QTOF-MS for <u>targeted</u> analysis of NPS with a test mixture of 40 NPS and metabolites in whole blood, urine, and oral fluid

Rebecca L Smith M.S.*, Anamary Tarifa, Ph.D., Anthony P. DeCaprio, Ph.D., Florida International University, International Forensic Science Research Institute, Miami, FL 33199.

Background/Introduction: To combat the rising use of NPS, laboratories have become more sophisticated in their screening and identification of compounds. While immunoassays are the preferred technique for screening common toxins, chromatographic separation coupled with mass spectrometry is the most preferred method for detection of NPS due to their selectivity, specificity, and sensitivity. LC-MS configurations include both low and high resolution via instruments such as LC-QqQ-MS and LC-QTOF-MS which can monitor specific precursor to product transitions for a specific compound. However, there is a lack of consensus in the potentials for these methods where a comparison is necessary for their use within forensics.

Objectives: This work provides the evidence to show the effectiveness of using LC-QqQ-MS and LC-QTOF-MS with data-dependent acquisition (DDA) for the analysis of multiple NPS. In this analysis, a 40 compound test mixture of NPS containing various structures and pharmacological classes was used to spike authentic matrices of whole blood, urine, and OF. The mixture included closely eluting NPS analytes, isomers, and metabolites that have a wide range in polarity, to allow for assessment of selectivity and other identification algorithms from the data obtained by each instrument. The performance of both acquisition methods was evaluated using figures of merit (LOD, dynamic range, linearity, selectivity, precision), carryover, and matrix effects.

Methods: Individual standard solutions of NPS were spiked in MeOH to create a mixture with a final concentration of 200 ng/mL for each compound. The mixture was further diluted with blank matrix to yield concentrations of 1, 2, 5, 10, 20, 50, and 100 ng/mL for determination of LOD, dynamic range, linearity, etc. for the targeted MS studies. Two extraction methods were used for specimens, dilute-and-shoot for urine and crash-and-shoot for whole blood and OF. The LC-QqQ-MS used a dMRM mode as a measure of selectivity, where specific MRM data was acquired to identify each compound based on their retention time, and transitions which included specific collision energies, fragmentor voltages, and product ion selections for each compound. The LC-QTOF-MS used a targeted MS/MS mode that specifically targeted the precursor, retention time, and collected a range of collision energies to confirm a compound. The fragmented product ions from the range of energies were analyzed in the TOF and both full scan and MS/MS data are acquired. Both instruments used positive and negative ESI ionizations for all of the compounds.

Results: The performance of these targeted methods was evaluated by their sensitivity, linear range, precision, matrix effects, and specificity in whole blood, urine, and OF. For spiked whole blood, a second approach was studied using a series of "dummy analytes" to mimic the presence of interferents in the matrix. Results were measured using statistical significance testing and a scoring system designed to summarize each analyte for each analytical method in positive and negative ESI modes. This was to present these methods as more effective in screening an evolving list of new substances that are difficult for older methods used in forensic laboratories.

P29 - Identification of multi-year trends of synthetic stimulants detected and identified by GC/MS at the Onondaga County Medical Examiner's Office

Samantha Starkey*, Kristie Barba, Onondaga County Medical Examiner's Office Forensic Toxicology Laboratory, Syracuse, NY 13210.

Background/Introduction: Over the past 6 years, cases reporting "Molly" use have increased but are negative for amphetamine based compounds detected by ELISA. Synthetic stimulants are predominately cathinone-based, which do not cross-react with amphetamine/methamphetamine kits, but are easily detected by GC/MS. Synthetic cathinones/stimulants interact with serotonin, dopamine, and norepinephrine transporters causing an increase availability of the neurotransmit-

ters, and are therefore widely abused for their effects of euphoria, decreased inhibition, increased libido, increased mental stimulation and energy. The adverse effects can include delusions, paranoia, tachycardia, hypertension, hyperthermia, and violent behavior.

Objectives: Identify multi-year trends of synthetic stimulants detected and identified by GC/MS. Compare synthetic stimulant trends to trends of common illicit drugs.

Methods: Case samples are routinely screened using ELISA and liquid-liquid extraction with GC/MS analysis. All ELISA results, except cannabinoids, are confirmed by SPE or LLE extraction and analyzed by GC/MS or LC/MS/MS. Synthetic stimulants are screened by using a LLE for alkaline drugs and analyzed by GC/MS scan with manual matching to MS libraries. When the calculated relative retention time (RRT) is within 0.05 of the known standard and the library match contains all diagnostic ions, the analyte is reported positive. Cases with possible synthetic stimulants that have not been identified by current libraries, or do not have a RRT, were tracked in a LIMS system. Once libraries were updated and new drug standards were available, these cases were re-evaluated to determine the identity of the synthetic stimulants.

Results: Synthetic stimulants were detected in 363 cases with a total of 433 substances found.

Synthetic stimulants found in casework								
	2016	2017	2018	2019	2020	2021	2022 (Jan-Jun)	Total (n = 433)
Alpha-PVP	1				1			2 (0.46%)
Butylone	11	3						14 (3.2%)
Dibutylone	14	3						17 (3.9%)
N,N-dimethylpentylone (DMP)						15	45	60 (13.9%)
Ethylone	1	1						2 (0.46%)
Eutylone			2	51	86	91		230 (53.1%)
Methylone				5				5 (1.15%)
N-ethylpentylone (NEP)	2	22	27	4				55 (12.7%)
Pentylone	1	1				10	36	48 (11.1%)

Total cases breakdown							
	2016	2017	2018	2019	2020	2021	2022 (Jan-June)
# cases	1278	1160	1150	1221	1380	1364	560
syn. stimulants (SS)	17 (1.3%)	23 (1.9%)	29 (2.5%)	55 (4.5%)	86 (6.2%)	107 (7.8%)	46 (8.2%)
Only*	8 (47.0%)	13 (56.5%)	17 (58.6%)	21 (38.1%)	27 (31.3%)	20 (18.6%)	7 (15.2%)
Cocaine	94 (7.3%)	90 (7.7%)	74 (6.4%)	94 (7.6%)	119 (8.6%)	136 (9.9%)	70 (12.5%)
with SS	4 (4.2%)	4 (4.4%)	6 (8.1%)	7 (7.4%)	12 (10.0%)	22 (16.1%)	11 (15.7%)
Fentanyl	175 (13.6%)	147 (12.6%)	156 (13.5%)	234 (19.1%)	291 (21%)	342 (25%)	134 (23.9%)
with SS	6 (3.4%)	6 (4.0%)	10 (6.4%)	31 (13.2%)	52 (17.8%)	75 (21.9%)	30 (22.3%)
Methamphetamine	15 (1.1%)	15 (1.2%)	11 (0.9%)	28 (2.2%)	61 (4.4%)	62 (4.5%)	29 (5.1%)
with SS	2 (13.3%)	1 (6.6%)		2 (7.1%)	11 (18.0%)	21 (33.8%)	8 (27.5%)

* = cases are negative for cocaine, fentanyl, and methamphetamine

Conclusion/Discussion: Overall prevalence of synthetic stimulants and poly drug use has rapidly increased in the last 6 years. It's unclear whether the rise in mixed drugs is attributed to the consumer demand or the impure nature of street drugs. As new drug standards are released, previously unknown synthetic stimulants can be identified by updating the in-house library and RRT log. Although the current method is not ideal for other classes of novel psychoactive substances, liquid-liquid extraction and GC/MS works well for identifying new synthetic stimulants found in case work.

P30 - Bromazolam concentration profile in Canadian post-mortem cases

Sandrine Mérette*, Sébastien Thériault, Lei Piramide, Angela Kim, Aaron Shapiro. Provincial Toxicology Center, Vancouver, BC, Canada.

Background/Introduction: Bromazolam, brominated equivalent of alprazolam, belongs to the benzodiazepine drug class, used for the treatment of a variety of disorders such as anxiety, panic disorder, insomnia and skeletal muscle spasticity. Detection of benzodiazepines with opioids in post-mortem samples has risen in recent years in British Columbia, Canada with etizolam and flualprazolam being the most frequently detected benzodiazepines (30 and 17% of illicit drug deaths respectively). Pharmacological activity of bromazolam was established in 1979 and was found to be active at the sub-nanomolar range at different GABA receptors, resulting in sedative, anxiolytic, anti-convulsing and muscle relaxant effects. There is a scarcity of literature on the pharmacological and toxicological effects of bromazolam hence its activity is usually compared to alprazolam, a well-known medication with high abuse potential and severe withdrawal symptoms. This study focuses on the detection, confirmation, and quantitation of bromazolam in post-mortem blood samples collected and analysed in 2021 and 2022 in British Columbia, Canada. The co-occurrence of benzodiazepines with opioids in post-mortem samples is also discussed.

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

Methods: Postmortem samples obtained from cases of suspected illicit drug toxicity were screened by LC/HRMS as part of routine post-mortem screening. Data files were retrospectively analyzed for the presence of bromazolam. Standard addition was used to quantitate bromazolam due to its recent emergence and relatively low prevalence. Samples were extracted by salt-assisted liquid-liquid extraction with ice-cold methyl tert-butyl ether/acetonitrile mixture and 3M ammonium acetate. After evaporation and reconstitution, the extracts were analysed using an Agilent 1290 LC system coupled with an Agilent 6470 triple quadrupole mass spectrometer (Agilent Technologies, Santa Clara, CA, USA). Linearity was evaluated (0.5-50 ng/mL) as well as the limit of detection (LOD), recovery, matrix effects, carryover from analyte or internal standard and commonly encountered drugs, including but not limited to opioids, over-the-counter medications, benzodiazepines, stimulants, Z-drugs, anti-depressants, and anti-psychotics (n=177). Controls were also prepared at two different concentrations (5 and 25 ng/mL) in blank blood. Alprazolam-d5 was used as internal standard.

Results: Bromazolam was detected, confirmed and quantitated in 41 postmortem cases in 2021 and 38 cases in 2022 (January to May) in British Columbia. Bromazolam concentrations ranged from 0.5 to 319.3 ng/mL, with a mean (\pm SD) bromazolam concentration of 11.4 \pm 53.7 ng/mL in 2021. From January to May 2022, bromazolam concentrations ranged from 2.5 to 1293 ng/mL, with an average concentration of 59.8 \pm 206.8 ng/mL. Bromazolam was confirmed in 2.8% of postmortem cases between January 2021 and May 2022. Bromazolam was commonly observed with other benzodiazepines, opioids (fentanyl, carfentanil), stimulants (cocaine, methamphetamine) or ethanol.

Conclusion/Discussion: Bromazolam is another illicit benzodiazepine that could become a public health issue, as its detection in medico-legal death investigations continues to increase. In this study, bromazolam was combined with an opioid (fentanyl, carfentanil or both) in the majority of cases. Bromazolam was mostly observed at very low concentrations in 2021. However bromazolam concentrations in post-mortem case samples are increasing in 2022. Combinations of several benzodiazepines were also observed in several cases, with etizolam and flualprazolam being the most detected. Potential additive pharmacological and toxic effects of these benzodiazepines combinations may increase drug overdose risks and lead to more deaths. Laboratories should consider adding bromazolam to their testing scopes due to its increasing prevalence in post-mortem investigations.

P31 - Quantitative analysis of designer benzodiazepines in human urine by LC-MS/MS

Shahana Huq*, Stephanie Marin, Danny Buchan

Phenomenex, Inc., Torrance, CA.

Background/Introduction: Benzodiazepines are among the most often prescribed drugs in US with significant abuse potential. Analogs to the prescription class of benzodiazepines, often obtained on illicit marketplaces, are not U.S. Food and Drug Administration (FDA) approved and are increasingly finding its place among the population over the last two decades. These designer benzodiazepine substances retain the core structure of the approved benzodiazepines; however, some functional groups are altered. This allows them to evade legal restrictions while keeping the desired mind-altering effects.

It is important to be able to track these substances as they arise so they can be monitored and detected. Due to cross reactivity of these analogs with Benzodiazepine immunoassay kits, developing effective LC-MS/MS techniques is increasingly important for proper identification and quantitation. In this study, we focused on 14 compounds in urine matrix utilizing a high sensitivity fast (less than 4 minutes) LC analysis. The panel comprises of 13 designer benzodiazepines. Alprazolam was also included because Deschloroetizolam is isomeric to it, and differentiation is necessary. Solid phase extraction (SPE) was used for sample cleanup to achieve a low limit of detection. A high efficiency 2.6 m, 50x3.0 mm Kinetex Biphenyl LC column was used for chromatographic separation, coupled with a SCIEX 4500 triple quad for MS/MS detection.

Objectives: The objective of this work is to develop a fast and reliable quantitation method for the detection of the targeted 14 benzodiazepines in urine.

Methods:An aliquot of 200 mL of urine sample spiked with 20 mL of internal standard (0.5 mg/mL) was loaded on a Strata-X Drug B Plus, 30 mg 96 well plate. The sample was treated with 60 mL of room temperature (RT) hydrolysis buffer, 20 mL of IMCSzyme RT enzyme and was incubated at RT for in-well hydrolysis following vortex or mixing for 20-30 seconds. After applying 2 successive washes with aqueous formic acid and 30% methanol (aq, v/v), the sample was eluted with ammoniated solution comprising of ethyl acetate and isopropanol in the ratio of 70:20:10 (ethyl acetate/isopropanol/ ammonium hydroxide).

Results: The optimized SPE method is far superior in terms of % CV and cleanliness compared to a variety of extraction methods evaluated. The absolute recovery obtained from Strata-X Drug B Plus ranged from 76-114% with a % CV values below 15%. Calibration curve constructed for 10³ fold dynamic concentration range (0.1 ng/mL-100 ng/mL) demonstrated linear regression values (R²) greater than 0.997 for all analytes using a quadratic fit with 1/x weighting factor. The three levels of QC showed accuracy and precision of 96-114% and <10% respectively.

Table 1. List of benzodiazepine drug panel and deuterated internal standards used.

Adinazolam
Bromazolam
Clonazolam
Deschloroetizolam
Diclazepam
Etizolam
Flualprazolam
Flubromazepam
Flubromazolam
Flunitrazolam
Methylclonazepam
Nitrazolam
Phenazepam
Alprazolam
Diclazepam-D4
Etizolam-D3
Etizolam-D3 Phenazepam-D4
Etizolam-D3 Phenazepam-D4 Clonazolam-D4

Conclusion/Discussion: We corroborated a sensitive, accurate and precise quantitation method for designer benzodiazepines, streamlining the workflow that accommodates for the hydrolysis of urine and SPE extraction in the same well of the Drug B plus plate. The integration of Strata-X Drug B Plus with in-well hydrolysis and fast LC method using the Kinetex Biphenyl Column creates an efficient workflow for expanding class of novel psychoactive substances.

P32 - Emergence of the nitazene class of novel synthetic opioids in postmortem toxicology and detection by LC-QToF-MS

Stuart A. K. Kurtz, M.S.^{1*}, Kevin G. Shanks, M.S.¹, George S. Behonick, Ph.D.^{1.1}Axis Forensic Toxicology, Indianapolis, IN 46268.

Background/Introduction: A new subclass of novel synthetic opioids (NSOs) has emerged since the scheduling of fentanyl analogs in 2018-2019. Termed nitazene analogs, or more simply, nitazenes, they are a benzimidizol-2-one structure that are distinct from other NSOs observed in case reports. Pharmacologically, they are similar to other opioids as mu opioid receptor agonists. Etonitazene was first synthesized in 1957 for research as a veterinary anesthetic, but was never approved. In 2019, isotonitazene was the first of the nitazenes to be detected in street drug seizures. Pharmacokinetic studies are limited and primarily focused in animal models. Relative potency in these animal studies determined nitazenes to be 100-1000 times more potent than morphine. The two groups that are estimated to be the most important for activity are the 5-nitro and para-benzyl groups.

Objectives: The rise of nitazene analogs prompted the addition of several compounds to the scope of our comprehensive testing in postmortem blood specimens. We surveil the following nitazene compounds: Butonitazene, etodesnitazene, etonitazene, flunitazene, isotodesnitazene, isotonitazene, metodesnitazene, metonitazene, N-pyrrolidino etonitazene (NPE), and protonitazene. The casework from our laboratory from June 1, 2021 – May 1, 2022 was examined for these nitazenes and together with substances concomitantly detected. Case history was provided by the client. Cause of death determinations that were certified by medical examiners/coroners are not available for these cases.

Methods: Postmortem blood specimens were sent to our lab in vials containing sodium fluoride as a preservative. Targeted comprehensive screening was performed after an acetonitrile protein precipitation extraction by liquid chromatography quadrupole time of flight mass spectrometry (LC-QToF-MS). Volatiles screening and confirmation was performed using head space gas chromatography with flame ionization detection (HS-GC-FID). Subsequent confirmation was done utilizing LC-QToF-MS, liquid chromatography with triple quadrupole mass spectrometry (LC-MS/MS), or gas chromatography with mass spectrometry (GC-MS). The nitazenes were reported qualitatively by LC-QToF-MS analysis with a limit of detection of 1 ng/mL.

Results: Nitazenes were detected in postmortem blood in 128 cases during the period of June 1, 2021 – May 1, 2022 across 8 states: Florida, Illinois, Indiana, Michigan, Nebraska, Ohio, Texas, and Wisconsin. Metonitazene (n=102) was the most common nitazene observed followed by isotonitazene (n=22), NPE (n=13), and flunitazene (n=7). Other common substances found with the nitazenes included: Fentanyl (n=106), 4ANPP (n=95), acetylfentanyl (n=41), naloxone (n=41), methamphetamine (n=38), delta-9-THC/delta-9-THC carboxylic acid (n=27), cocaine/benzoylecgonine (n=26), morphine (n=25), and fluorofentanyl (n=25). Other NPSs detected included brorphine (n=4), flualprazolam (n=4), etizolam (n=3), butyrlfentanyl (n=3), and acrylfentanyl (n=1).

Conclusion/Discussion: We detected 4 different nitazenes (metonitazene, isotonitazene, NPE, and flunitazene) in 128 cases from June 1, 2021 – May 1, 2022. Other substances detected in combination with the nitazenes included: Fentanyl, 4ANPP, acetylfentanyl, naloxone, and methamphetamine. Forensic toxicology laboratories should consider including nitazenes in their testing strategies and continuously monitor geographical/regional trends for emerging NSOs in the jurisdictions they serve.

P33 - Determination of novel psychoactive substances (NPS) and synthetic opioids in meconium

Theresa Lee^{1*}, Pierre Negri¹, Ángela López-Rabuñal², Daniele Di Corcia³, Eleonora Amante³, Marta Massano^{3,4}, Angelines Cruz-Landeira², Ana de-Castro-Ríos², Marco Vincenti^{3,4}, Alberto Salomone^{3,4}.¹SCIEX, Redwood City, CA, USA; ²Servizo de Toxicoloxía, Instituto de Ciencias Forenses, Facultade de Medicina, Universidade de Santiago de Compostela, Santiago de Compostela, Spain; ³Centro Regionale Antidoping e di Tossicologia "A. Bertinaria", Orbassano, Turin, Italy; ⁴Dipartimento di Chimica, Università degli Studi di Torino, Turin, Italy.

Background/Introduction: The widespread emergence of NPS in the recreational drug market constitutes a public safety threat worldwide, as these substances vary greatly in purity and potency. Of the various populations exposed to NPS, pregnant women are among the most vulnerable to the harmful effects of these substances. Meconium, which is a newborn's first stool, is considered the best matrix for detecting prenatal drug exposure. Although complex in composition, meconium provides a wide detection window that covers the second and third trimesters of pregnancy. Therefore, reliable screening methods capable of identifying and quantifying drug use in meconium samples provide unequivocal evidence of prenatal exposure to NPS and synthetic opioids.

Objectives: The objective of this study was to develop a fast, reliable, and quantitative screening workflow for the detection of 137 NPS in meconium.

Methods: A total of 137 NPS including 54 synthetic cannabinoids and metabolites, 49 synthetic cathinones, stimulants, dissociatives and hallucinogens, 34 fentanyl analogs and synthetic opioids were targeted in this workflow. Blank and authentic meconium specimens spiked with various concentrations of the 137 NPS were homogenized and subjected to solid-phase extraction (SPE). Analytes were chromatographically separated using a Phenomenex C18 column (100 x 2.1 mm, 1.7 μm, 00D-4475-AN) held at 45 °C. Mobile phase A (MPA) and mobile phase B (MPB) consisted of water with 0.1% formic acid, and acetonitrile with 0.1% formic acid, respectively. MS and MS/MS data were collected for each sample using SWATH acquisition on the SCIEX X500R QTOF system in positive mode. Data acquisition was TOF MS scan followed by 12 MS/MS scans using variably sized Q1 windows, covering a mass range from 150 to 465 Da.

Results: A series of blank meconium samples spiked with the 137 NPS were injected at eight concentration levels to assess the quantitative performance of the developed method. The calibration curves showed excellent linear responses across the calibration series, with R² values greater than 0.99 for the wide majority of the NPS targeted in the panel. The ability to reproducibly quantify drugs and metabolites extracted from meconium samples was investigated by performing 6 consecutive injections and calculating the average %CV value for each of the 137 molecules. These %CV values were consistently below 20%, indicating that this screening workflow using the SCIEX X500R QTOF system is capable of precise quantification. The robustness and quantitative performance of the screening workflow was further investigated by analyzing 30 authentic meconium specimens from newborns. Four meconium specimens tested positive for fentanyl at concentrations ranging from 440 to 750 ng/g and 2 specimens tested positive for acetylfentanyl at concentrations ranging from 190 to 1400 ng/g.

Conclusion/Discussion: A quantitative screening workflow for the detection of 137 NPS extracted from meconium is described. The combination of a selective sample preparation method, including homogenization followed by solid phase extraction (SPE), and the use of SWATH acquisition on the SCIEX X500R QTOF system enabled robust quantification of drugs and metabolites with a wide range of physical and chemical properties. The results of the analysis of the 30 authentic meconium specimens demonstrate the robustness of the presented workflow and its ability to reliably screen NPS and synthetic opioids from meconium.

P34 - Nitazenes at the Roxbury: an analytical workflow for the analysis of emerging synthetic opioids of the nitazene class

Jennifer Hitchcock^{*1}, **Timothy Bolduc**¹. ¹Agilent Technologies, Lexington, MA.

Background/Introduction: Isotonitazene is the most frequently encountered member of the 2-benzylbenzimidazole opioids class of compounds, often referred to as nitazenes. It is a structural analog of etonitazene which was first synthesized in the 1950s. N-pyrrolidino etonitazene (etonitazepyne) is a high potency synthetic opioid which has recently emerged on the illicit market. Unlike other nitazenes, etonitazepyne has not been reported in previous literature or patents. These compounds are approximately 20x more potent than fentanyl and pose significant risks health risks. There is a need for accurate, sensitive analytical methods for the detection of nitazene analogs.

Objectives: The primary objective of this work was to develop an end-to-end quantitative workflow using triple quadrupole LC/MS for the analysis of emerging synthetic opioids in the nitazene class, specifically N-pyrrolidino etonitazene.

Methods: Commercially available blank whole blood was fortified with drug standards from a working stock solution, and a 1 mL aliquot was loaded onto the Captiva EMR-Lipid filtration cartridge. Each sample was treated with an acetonitrile:methanol mixture containing the isotopically labeled internal standard for an *in situ* protein precipitation. Low pressure was applied to pull the samples through the sorbent for pass-through sample cleanup. After all cartridges appeared dry, an aliquot of 80/20 ACN/water was added for additional elution. High-level pressure was applied to dry the sorbent bed completely. The eluate was dried under nitrogen at 40°C for 15 minutes. Samples were reconstituted with mobile phase starting conditions and analyzed by triple quadrupole LC/MS.

Results: The two analytes monitored were etonitazepyne and isotonitazene, as well as isotopically labeled isotonitazene for the internal standard. These compounds were chromatographically resolved on an EC-C18 column (2.1x100 mm, 2.7 μm) with a 6.5-minute gradient using an Agilent 1290 Infinity II binary pump and a 6470B triple quadrupole. Excellent linearity and reproducibility were demonstrated over a concentration range of 0.1 ng/mL to 100 ng/mL, with a correlation coefficient of greater than 0.999 for both analytes. All calibration levels showed variability of less than 5%, and each compound had an estimated limit of detection of 0.1 ng/mL, while the limit of quantitation for each is 0.5 ng/mL in whole blood.

POSTER

Conclusion/Discussion: The Captiva EMR-Lipid workflow was designed to selectively remove lipids and additional matrix components without analyte loss using a unique combination of size exclusion and hydrophobic interaction mechanisms with the sorbent material. This sample preparation method provides simple and efficient analyte extraction and matrix cleanup. The estimated LODs and LOQs in whole blood closely matched previously reported concentrations using different sample preparation methods. Etonitazepyne has a molecular weight that differs from previously identified nitazenes, which allows for easy addition to LC-MS/MS methods. Nitazenes are not routinely tested for in post-mortem or urine toxicology; therefore, it is important to develop and update analytical methods. An end-to-end workflow was developed for the detection and quantification of nitazene analogs using an Agilent 6470B triple quadrupole mass spectrometer.

P35 - Assessment of recombinant beta-glucuronidase vs native beta-glucuronidase and alkaline conditions for the hydrolysis of THC-carboxylated metabolite glucuronide

Alberto Salomone^{*1,2}, Enrico Gerace². ¹ Department of Chemistry, University of Turin, Italy. ² Centro Regionale Antidoping, Orbassano (TO), Italy.

Background/Introduction: The use of enzymes plays an important role in the hydrolysis of conjugated molecules during the preparation of biological samples, especially urine. Several clinical and forensic applications, including Therapeutic Drug Monitoring (TDM) and toxicological analyses, can take advantage of an effective, easy, time-saving procedure.

Objectives: In this perspective, we aimed to assess the yield of two different beta-glucuronidase enzymes, one recombinant and one native, in comparison with the alkaline hydrolysis routinely used in our laboratory for the analysis of THC in urine samples.

Methods: A total of 82 anonymous urine samples, collected from individuals who had given informed consent to be tested for drugs of abuse, were tested with an ISO 17025 accredited UHPLC-MS/MS method for the detection of THC-carboxylated metabolite. During the sample preparation, three different pre-analytical hydrolysis steps were performed: (A) 100 μ L of urine was added with 4 μ L of NaOH 10N, then incubated at 55°C for 15 minutes; (B) 100 μ L of urine was added with 200 μ L of recombinant beta-glucuronidase (rec- β -gluc), then incubated at room temperature for 5 minutes; (C) 100 μ L of urine was added with 10 μ L of native beta-glucuronidase from *Helix Pomatia* (nat- β -gluc), then incubated at 55°C for 15 minutes. In a second experiment, further 37 anonymous urine samples were tested with different volumes (respectively, 20, 50 and 100 μ L) of rec- β -gluc, to optimize the working conditions.

Results: According to the concentrations obtained with the routine procedure, the 82 samples were divided into "high concentration" (HC, above 55 ng/mL) and "low concentration" (LC, below 55 ng/mL) samples. For the HC samples, the yield obtained using rec- β -gluc was on average 13.7% higher than alkaline hydrolysis and 23.4% higher than nat- β -gluc procedure. For the LC samples, the yield with rec- β -gluc was on average 12.4% higher than alkaline hydrolysis and 15.5% higher than the nat- β -gluc procedure. A Wilcoxon test was run, and all procedures proved significantly different. The second experiment showed that the THC-carboxylated metabolite concentrations were not significantly different using the three different volumes of rec- β -gluc, proving that a minimum amount of 20 µL is enough to obtain the highest hydrolysis efficiency.

Conclusion/Discussion: The recombinant beta-glucuronidase provided the most efficient hydrolytic activity to cleave the glucuronide of THC-carboxylated metabolite. Further experiments are needed to verify that similar results are obtained for other glucuronate metabolites, including pharmaceuticals and other cannabinoids, primarily cannabidiol. By achieving a high yield in only 5 minutes without a heating step, the laboratories, especially in clinical, forensic, TDM, and workplace drug testing, will be enabled to increase the number of processed urine samples, with no detriment of the analytical performances.

P36 - Raising the quality of initial post-mortem screening to the next level by LC-MS/MS merged product ion scanning

Alexander D. Giachetti^{*}, Jacob M. Samuel, M. Elizabeth Zaney, Joseph H. Kahl, George W. Hime, Diane M. Boland. Miami-Dade Medical Examiner Toxicology Laboratory, Miami, FL.

Background/Introduction: The Miami-Dade Medical Examiner Toxicology Laboratory processes more than 3,000 post-mortem cases per year, beginning with a routine blood drug screen by GC-MS and an immunoassay analysis. To improve the scope and quality of testing, adding additional screening tests were the norm which increased cost and turnaround time. Without access to unlimited funding and new instrumentation, the laboratory decided to explore the viability of replacing the current immunoassay system with a modern LC-MS/MS screening assay. This new method would transcend the current limitations of immunoassay and serve as a complement to the laboratory's GC-MS blood drug screening.

Objectives: Develop and validate a method by LC-MS/MS fit for purpose into the laboratory's routine case workflow. It must prove to be more sensitive and specific than immunoassay, encompass a broad range of toxicologically relevant compounds, use a small sample volume, function appropriately when applied to post-mortem samples, and instill greater confidence in routine screening work.

Methods: 200µL of Post-mortem blood and tissue homogenates were fortified with pH7 phosphate buffer and 10 µL of internal standard before being vortexed, centrifuged, and loaded on to Biotage ISOLUTE SLE+ cartridges. 4mL 75:25 Hexane:Ethyl Acetate, 2mL Hexane and 2mL MTBE were used as elution solvents. 100µL of 1% Hydrochloric Acid in Methanol was added to the elution tubes prior to evaporation and reconstitution in 50µL 50:50 Mobile Phase A (0.1% Formic Acid in Water) and Mobile Phase B (Methanol). 2µL of sample was injected for instrument analysis with a Shimadzu LCMS-8060NX, triple quadrupole mass spectrometer. Chromatographic separation was achieved with a Restek Raptor Biphenyl 2.7µm 50x2.1mm. Mass spectral events were created for each compound, consisting of two MRM transitions. Additionally, "Survey Event Mode" was utilized for each compound, which collected three product ion scans at different collision energies from the precursor ion once a signal threshold from the MRM was reached. The three product scans were merged into a single spectrum and compared to an in-house library made from certified reference materials.

Results: Library entries were created for sixty compounds that cross-react with current immunoassay kits: benzodiazepines, amphetamines, benzoylecgonine, opiates, oxycodone, and cannabinoids. An internal standard representing each of these drug groups was present to ensure a consistent analysis. Frequently detected analytes including fentanyl and xylazine were also added to the library. Limit of detection for all compounds either met or exceeded cut-off concentrations from EMIT and ELISA immunoassays.

Over 100 post-mortem cases were submitted to the extraction procedure and analyzed blind prior to validation to evaluate the performance of the method. At least 90% of the cases analyzed on the new LC-MS/MS method agreed with the current workflow of immunoassay and GC-MS blood drug screen results. All false positives from amphetamines and benzodiazepines immunoassays, particularly in decomposed specimens, were eliminated when analyzed by LC-MS/ MS. The product ion scan library matching identified commonly missed analytes in the current routine workflow, such as amphetamines, THC, fentanyl, naloxone and low-level benzodiazepines. The resulting spectral detail also eliminated misidentifications often found when using MRM scanning alone. Analysis was simple and fast due to filtering results by triggered scans only.

Conclusion/Discussion: The method developed has proved efficient at extracting and detecting many of the drugs identified in postmortem casework as well as novel psychoactive substances and emergent compounds. Detection limits improved by a factor of 10 for most compounds. The scanning speed of Shimadzu instrumentation allows the collection of large amounts of data without compromising data quality, enabling product ion scan library matching rather than traditional multiple reaction monitoring. The blind case studies provided confidence that validation will be successful, and implementation will prove valuable to the laboratory.

P37 - A fast and green method for LSD urinalysis

Amy L. Patton^{*1}, Jordan Brown¹, Kimberley Heine^{1, 2}, David Sartori¹, Erin L. Karschner¹, and Jeffrey P. Walterscheid¹. ¹Division of Forensic Toxicology, Armed Forces Medical Examiner System, Dover AFB, DE. ² Forensic Toxicology Division, Washington DC Office of the Chief Medical Examiner, Washington, DC.

Background/Introduction: Lysergic acid diethylamide (LSD) has a long history of use in the United States, both for therapeutic and recreational purposes. It has been touted as a cure for a wide range of conditions such as schizophrenia, alcoholism, and psychosis, among many others. Recently, the resurgence of illicit LSD use and the introduction of LSD analogs into novel psychoactive markets has been the subject of news headlines around the world. For testing panels to remain relevant, workplace drug testing laboratories and drug deterrence programs should explore the addition of LSD and its metabolite to current testing menus. However, historical methods of extraction and analysis typically utilize large sample volumes, harmful solvents, and labor-intensive sample handling, which is too costly and inefficient for routine monitoring.

Objectives: After attending this presentation, attendees will be able to develop a high-throughput method to quantify LSD and its metabolite 2-oxo-3-hydroxy-LSD in urine, while achieving relevant sensitivity and specificity requirements.

Methods: LSD and 2-oxo-3-hydroxy-LSD were extracted from urine using RP-WAX Dispersive Pipette XTRaction (DPX) tips on a Hamilton STARlet Liquid Handling Workstation. Initially, the deck of the liquid handling system was prepared by placing the extraction solvents in appropriate locations. In order to make the method as efficient and ecofriendly as possible, pipette tips underwent in-batch recycling when possible. The automated handling system distributed internal standard to all calibrators, controls, and samples. The extraction tip was first conditioned with 30% methanol, then the specimen was drawn into the tip, washed with water, and then eluted with acetonitrile. All tubes were dried and reconstituted in 0.1% formic acid in deionized water and 0.1% formic acid in acetonitrile (90:10 v/v) prior to analysis by liquid chromatography tandem mass spectrometry (LC-MS/MS).

Results: The method was validated to Department of Defense Instruction (DoDI) 1010.16 standards and included studies such as linearity, bias, precision, limit of detection, lower limit of quantitation, interference, carryover, matrix effects, stability, and dilution integrity. Calibration curves for both analytes ranged from 0.05 – 5.0 ng/mL with limits of detection/ quantitation at 0.05 ng/mL. All other studies met or exceeded acceptability criteria. The method was authorized for confirmation/quantitation of random drug testing samples that previously screened presumptively positive by immunoassay at other drug monitoring facilities.

Conclusion/Discussion: This automated extraction method permitted rapid, sensitive, and robust confirmation and quantitation of LSD and its metabolite in urine specimens. Automation provides additional reliability for quality assurance and quality control measures such as proper test tube orientation through barcode reading, automated dilutions, error tracking, and weekly pipette channel calibration checks, while increasing efficiency. This simple extraction and instrument method permits easy incorporation of a number of analytes into any workplace drug testing program and allows for a more environmentally friendly process with smaller sample and waste volumes. An analytical method that reduces manual extraction steps, while achieving low detection limits for LSD and its primary urinary metabolite, 2-oxo-3-hydroxy-LSD, will be valuable to high-throughput laboratories seeking to add LSD to their scope of testing.

P38 - Best practices in urine drug hydrolysis methods

Ana Cabello*, Kura Biotech, Puerto Varas, Chile. Janet Jones, Kura Biotech, Los Angeles, CA. Nicholas Chestara, DPX Technologies, Columbia, SC. Camila Berner, Kura Biotech, Puerto Varas, Chile.

Background/Introduction: Best laboratory practices in urine drug hydrolysis methods are essential to ensure the robustness and harmonization of LC-MS/MS assays for clinical diagnostics. However, the method development and validation plans need to be tailored to the particular assay format being established.

Objectives: Thus, we aim to offer a simplified technical guideline intended for use in clinical and forensic laboratories. Based on the acquired expertise by Kura Biotech in the field of toxicology, we cover topics such as the ones mentioned in Methods.

Methods: 1) how to define your laboratory needs, 2) all you need to know about available enzymes and how to select the appropriate enzyme based on your method needs, 3) how to develop a proper protocol based on the drug class being tested, 4) how to design an enzyme comparative study.

Conclusion/Discussion: The present work states a fundamental approach needed to successfully optimize and validate methods pertaining to hydrolyzing drugs and their metabolites for urine drug testing

P39 - Detection of gabapentin in keratinized specimens

Andre Sukta*, Amy Racines, Joseph Jones. United States Drug Testing Laboratories, Inc., Des Plaines, IL.

Background/Introduction: Gabapentinoid drugs are effective in neuropathic pain. Gabapentin is used to treat partial seizures, nerve pain from shingles, and restless leg syndrome. Recently there are concerns about increased prescribing and the implications for patient safety, misuse, and diversion. In 2019, the Centers for Disease Control and Prevention noted that 69 million gabapentin prescriptions were dispensed in the United States. Prevalence of gabapentin misuse is 15-22% within populations who abuse opioids, 40-65% among individuals with prescriptions and approximately 1% in the general population as published in Addiction. Gabapentin is misused primarily for recreational purposes, self-medication, or intentional harm. It also was misused alone or in combination with other substances, especially opioids, benzo-diazepines, and/or alcohol. Keratinized specimens are a matrix of choice for a longer look back and/or compliance with an abstinence program. The novel screening technique used in this study was ideal for high throughput as it is able to analyze for multiple analytes in an 8 second run versus the 8-minute runtime for our confirmation analysis.

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

Methods: Keratinized specimens were weighed out (20 mg) and washed using acetone. The samples were homogenized in a Biospec Beadbeater-24, sonicated in methanol and the supernatants were subjected to an extraction procedure using a mixed mode solid phase column. The dried extracts were reconstituted with a methanol: deionized water solution containing 100 μ g/mL of EDTA. Extracts (8 μ L) were then spotted into a LazWell 96 Plate [Phytronix, Toronto, Canada] and dried prior to analysis on the LDTD-MS/MS with a runtime of 8 seconds per sample. Presumptive positive specimens were confirmed through a second aliquot (50 mg), washed, homogenized, sonicated in methanol. The supernatant was filtered, evaporated, and reconstituted in mobile phase prior to analysis by LC-MS/MS. The LC-MS/MS procedure used a 50 x 2.0 mm Hydro-RP with 2 μ m particle size, an 8-minute total run time, mobile phase A is 10mM Ammonium Acetate with 0.1% Formic Acid in water, mobile phase B Acetonitrile with 0.1% Formic Acid. Authentic specimens were chosen by screening random de-identified positive samples destined for destruction.

Results: The Standard Practices for Method Validation¹, was followed. The LDTD-MS/MS analysis had a cutoff of 200 pg/mg, with a limit of detection (LOD) of 100 pg/mg. The extracts and dried LazWell plates were stable for 48 hours, and no carryover or obvious interferences were observed during the validation. Significant ion enhancement (>25%) was observed. Authentic specimens were analyzed and presumptive positive specimens were forwarded to LC-MS/MS for confirmation.

The confirmation method was validated with a cutoff of 200 pg/mg and a LOD of 20 pg/mg. Precision and accuracy were challenged at 80 pg/mg (Low), 250 pg/mg (Mid) and 2000 pg/mg (High), and results were acceptable. Extracts were stable for up to 7 days, there was minimal ion enhancement (<25%) and no obvious interferences or carryover were observed. Linear range was determined to be between 40 pg/mg and 2000 pg/mg. All authentic specimen analyzed showed 100% correlation with the screening analysis.

Discussion/conclusion: Gabapentin has found an increased use and abuse among people who already have a prescription and for those who have previously abused opioids. The validated method described here allows accurate identification of usage of Gabapentin, allowing for objective proof that can be used by rehab and diversion programs to intervene and provide more successful outcomes. Recently a nonprofit group has filed a petition with the Food and Drug Administration and Drug Enforcement Agency to make the drug a controlled substance.

P40 - Evanescent wave technology applied to the detection of drugs in oral fluid

Pierre Cassigneul¹, Eric F Rieders^{1,2}, **Barry Logan***². ¹ Evanostics LLC, Horsham PA; ² NMS Labs, Horsham PA.

Background/Introduction: With the notable exception of ethanol, most point of contact testing (POCT) systems used for the rapid detection of the possible presence of one or more abused drug classes in biological fluids exploit the well-es-tablished chemistries of ligand binding assays (LBAs) and have historically utilized laminar flow technology (LFAs) with either visual or instrumented detection. The demand for such systems to detect drugs of abuse has until recently been driven by the widespread preference of urine as the specimen of choice in drug screening applications, and thus the well-known limitations of these diagnostic systems with respect to sensitivity have not until now generally limited their utility in practice. New approaches are required to achieve the sensitivity required for oral fluid testing.

Objectives: We describe the successful exploitation of planar waveguides for the simultaneous detection of multiple drug classes in oral fluid samples at reporting limits appropriate for both forensic and clinical applications.

Methods: The detection technology involves deposition of chemical biosensors on the surface of a silicon planar waveguide designed such that only the near-surface evanescent fields associated with excitation and emission are involved in the LBA. The LBA cartridge used tested for fentanyl, THC, cocaine, methamphetamine, 6-MAM, methadone, opiates, oxycodone, and benzodiazepines. Samples from 58 patients in a pain management clinic were tested on a prototype system. were compared to those obtained by LCMSMS.

Results: 42 out of 58 cartridges competed the rapid onsite test from samples provided by the patients. Samples screened and were confirmed positive for fentanyl (n=7), THC (n=1), methamphetamine (n=1), opioids (n=9), oxycodone (n=12), benzodiazepines (n=3), and buprenorphine (n=6). There were 39 true positives, 16 false positives, 5 false negatives, and 360 true negatives. Sensitivity ranged from 78% to 100%, specificity from 79% to 100%, and accuracy from 79% to 100%.

Conclusion/Discussion: This preliminary field assessment demonstrated the performance capabilities of evanescent wave technology for the detection of near-surface immunochemistry as a viable technology for sensitive, multiplexed oral fluid drug screening.

P41 - Natural inhibitors in urine can reduce glucuronidase performance and result in lowered recoveries

Claire Collins^{*1}, Amanda C. McGee¹, Caleb R. Schlachter¹, and L. Andrew Lee¹. ¹Integrated Micro-Chromatography Systems, Inc, Irmo, SC 29063, USA.

Background/Introduction: Urine drug testing is one of the most common practices for monitoring the use of prescribed opioid medications. Testing is typically performed by a preliminary screening assay, such as immunoassay, followed by a confirmatory assay such as liquid chromatography coupled with mass spectrometry (LC-MS/MS). Screening and confirmatory assays benefit from the use of enzymes that hydrolyze, or deconjugate, glucuronidated analytes. New generation of beta-glucuronidases can now effectively cleave glucuronides in urine at room temperature. However, during the studies, we have identified additional challenges in urine across biologically relevant pH extremes (4 or 9) and patient specimens.

Objectives: Screening and characterizations of new generation of beta-glucuronidases against varying concentrations of chemicals present in urine.

Methods: IMCSzyme RT was provided by Integrated Micro-Chromatography Systems (IMCS). BpGUS is a recombinant enzyme expressed in *E. coli* with gene sequence sourced from *Brachyspira pilosicoli*. The enzyme is purified by immobilized metal affinity chromatography (IMAC) followed by buffer exchange. Both enzymes are approximately 2 mg/mL of protein.

Enzyme activities are measured using a substrate-metabolite cross titration assay where substrate and metabolite concentrations varied while enzyme concentration remain constant. Hydrolysis rates are calculated and plotted as a function of substrate concentration and hydrolysis rate at each metabolite concentration. 4-methylumbelliferyl glucuronide hydrolyzes to 4-methylumbelliferyl and can be measured with fluorescence. Substrate and metabolites were mixed, and hydrolysis was initiated with BpGUS addition to substrate-metabolite mixture. Reaction was quenched in 20 second intervals for 120 seconds.

Opioid-positive urine specimens were obtained from a national testing laboratory. Negative controls and patient specimens were buffered and hydrolyzed with IMCSzyme RT or BpGUS. Hydrolyzed urine samples were cleaned with solid phase extraction, solvent evaporated, reconstructed in methanol and diluted with mobile phase A (0.1% formic acid in water). 10 µL of sample was injected on a Thermo Scientific[™] Vanquish[™] UHPLC system coupled with a Thermo Scientific[™] Endura[™] Triple Quadrupole Mass Spectrometer using a Phenomenex Kinetex[®] **2.6** µm Phenyl-Hexyl 100 Å, 50 x 4.6 mm column. Mobile phase B was 0.1% formic acid in acetonitrile.

Results: BpGUS activity drops approximately 15% at urea level of 25 mM or 70 mg/dL and drops nearly 40% when urea concentration is 100 mM or 350 mg/dL while the activity of IMCSzyme RT activity drops approximately 10% and 30% at the same urea concentrations. Well-known inhibitor D-saccharic acid 1,4-lactone monohydrate has a Ki value of 0.001 mM towards BpGUS, which is one of the lowest inhibition concentrations reported thus far for a glucuronidase. Other natural metabolites inhibiting this enzyme show higher Ki values ranging between 40-50 μ M, and other common metabolites from coffee, vitamin C, and glucuronic acid did not have a significant impact on either enzyme. The potential negative impact is highlighted with six patient samples that exhibit varying degrees of inhibition. In one patient out of six, oxymorphone recovery is incomplete despite the addition of a large quantity of enzyme.

Conclusion/Discussion: Not all enzymes have the same tolerances toward inhibitors in urine. One approach to avoid the complexity of natural inhibitors or urea reducing the hydrolysis efficiency is to dilute down the urine to lower the urea concentration and the concentration of the natural inhibitors. Such dilution is also beneficial in achieving the target pH for optimal hydrolysis, whereas limited dilution or buffer addition can result in reduced pH control. Heterogeneity of urine is a common fact, even if sourced from a single patient, the hydration levels and food metabolites will significantly vary. Although further dilution of urine will dilute target analytes, and possibly require a higher instrument sensitivity, there will always be economical and practical balancing act of efficient enzymatic hydrolysis.

P42 - Reproducibility of a volumetric absorptive microsampling device for quantitation of drugs of abuse in blood

Michael Roberts¹, **Colin Bunner**^{*1}, Madison R. Malone¹, Frederic Ciner¹. ¹Chem Quant Analytical Solutions, LLC, Apex, NC.

Background/Introduction: Amongst alternative matrices for clinical testing, dried blood is particularly appealing due to shipping/storage considerations and the possibility of sample collection outside of traditional collection environments. A key concern for clinical testing of samples collected outside of a controlled environment is reproducibility, a concern which has thus far limited the adoption of dried blood analytical techniques. In previous work, we demonstrated the reproducibility of a Volumetric Absorptive Microsampling (VAMS®) device, namely Neoteryx's Mitra® device, for quantitation of cannabinoids and endogenous hormones in spiked whole blood. Here, we extend that work to a toxicology panel covering 33 representative drugs of abuse. The selected analytes cover opioids, opioid antagonists, novel hallucinogens, amphetamines, antipsychotics, benzodiazepines, fentanyl/fentanyl analogues, barbiturates, and an alcohol metabolite. By dipping Mitra® tips in spiked whole blood and repeating analyte quantitation via LC/MS/MS, sample reproducibility was determined.

Objectives: Determine the reproducibility of a VAMS[®] device for the accurate and precise quantitation of drugs of abuse in blood.

Methods: Whole blood spiked with analytical standards was used to dip the Mitra[®] tips according to manufacturer instructions. The dried tips were extracted and analyzed on a Shimadzu Nexera autosampler coupled to a Shimadzu LCMS-8060 triple quadrupole mass spectrometer. Analytes were quantitated using a 7-point linear calibration curve with concentration-based weighting. Three quality control levels were used to assess the accuracy of the model. Separately, 4 independently prepared samples at the mid-level quality control (QCM) level were injected a total of three times each to assess intra- and interday accuracy and precision. The linear range varied by analyte, with the lowest calibration points at 0.25 ng/mL and the highest at 3,750 ng/mL.

Results: All analytes in our panel demonstrated high accuracy and low sample variance across repeated sampling, with accuracies for the individual QCM samples under 20% and CVs across the repeat QCM injections under 20%.

Conclusion/Discussion: Our data demonstrate that the Mitra[®] tip is a reliable device for the quantitation of drugs of abuse in whole blood. The variance between samples is low enough for clinical application of this device. Future work is needed to assess the reliability when applied to finger prick samples.

P43 - Cutoff concentration evaluation of a fentanyl screening assay

Dan Wang*1, Randal Schneider2, Shelby L Cunningham1.

¹Department of Pathology and Laboratory Medicine, Beaumont Health System, Royal Oak, 3601 W 13 Mile Road, Royal Oak, MI 48073. ²Abbott Diagnostics, Research & Development-Scientific Affairs, 100 Abbott Park, Abbott Park, IL 60014.

Background/Introduction: With the surge of Fentanyl-related overdose deaths in recent years, screening for fentanyl has been adopted by many clinical laboratories to detect illicit drug use and monitor compliance. Immunalysis[®] **SEFRIA™ Fentanyl Urine Enzyme Immunoassay** is the first FDA-cleared fentanyl enzyme immunoassay for clinical diagnostic use. However, recent studies based on real patient specimens have revealed high false-positive rates of this screening assay.

Objective: To evaluate the FDA-approved cutoff of SEFRIA[™] Fentanyl Urine Enzyme Immunoassay on Abbott ARCHITECT chemistry analyzers, and derive a new cutoff based on the study patient population.

Methods: 20 urine samples, obtained from two reference laboratories of known fentanyl and norfentanyl concentrations were screened using the assay cutoff of 1.0 ng/mL per the package insert. Among the 20 samples, two samples have undetectable fentanyl (<1.0 ng/mL) and norfentanyl (<1.0 ng/mL), two samples have undetectable fentanyl (<1.0 ng/mL) but detectable norfentanyl (7.3 and 8.3 ng/mL), and 16 samples have fentanyl concentrations ranging from 0.4 ng/mL to 24 ng/mL. All fentanyl and norfentanyl concentrations were determined by liquid chromatography-tandem mass spectrometry (LC-MS/MS) using cutoffs of 1.0 ng/mL except fentanyl from one reference lab that using 0.2 ng/mL as the cutoff.

Results: During initial screens using a cutoff of 1.0 ng/mL, the assay was positive for all 16 samples with detectable fentanyl (0.4-24 ng/mL). Of the two samples with undetectable fentanyl (<1.0 ng/mL) and detectable norfentanyl, one was positive (norfentanyl 7.3 ng/mL) and the other was negative (norfentanyl 8.3 ng/mL). Positive results were obtained for two samples with undetectable fentanyl (<1.0 ng/mL) and norfentanyl (<1.0 ng/mL).

Based on these results, a retrospective review of results from July 7, 2020 to August 30, 2020, was performed to assess the impact of possible false positive rate. A total of 410 fentanyl confirmations were performed by LC-MS/MS at Beaumont laboratory, and 155 samples (38%) were identified as false positive. In review of all the false positive screens, 59% of the screening results were in the range of 1.0 - 1.3 ng/mL. For all the positive screens (false positive plus confirmed positive), 30% of the screening results fall between 1.0 and 1.3 ng/mL.

Conclusion/Discussion: The SEFRIA fentanyl screen is calibrated with fentanyl only by design and therefore has a poor cross-reactivity with norfentanyl (0.0050%), which may explain the false negative result for the sample with detectable norfentanyl. For false positive results obtained for two samples with undetectable fentanyl and norfentanyl, the presence of designer fentanyl analogues could not be excluded.

To reduce the number of false positive screens, the assay cutoff was increased from 1.0 ng/mL to 1.3 ng/mL. The increase in cutoff concentration may result in low-positive patients being missed during screening. However, low positive results are generally observed in patients prescribed with fentanyl, while illicit drug use typically reflects a high-positive screen result in our experience. Taking both clinical impact and laboratory workload into consideration, the new cutoff is appropriate for the detection of illicit fentanyl use in the study patient population and have provided education to health care providers impacted by this change.

P44 - Withdrawn

P45 - Automated solid phase extraction as-compared-with manual vacuum manifold sample preparation of amphetamines in keratinized matrix analyzed by LC/MS/MS

Dominique Gidron*¹, Graham Kennedy¹, Donna Coy¹, Joseph Jones¹. ¹United States Drug Testing Laboratories, Inc, Des Plaines, IL USA.

Background/Introduction: Solid Phase Extraction (SPE) is the go-to method for sample preparation throughout a variety of industries, including Forensic Toxicology. The technique allows analytical chemists to concentrate analytes of interests, remove matrix interferences and increase analytical power. SPE methods are broad and limitless; however, manual extractions present workflow challenges such as intensity of labor, large time consumption and risks of errors. To alleviate the challenges of this process while maintaining a high quality of analytical results, this abstract explores the use of an automated SPE instrument, Biotage[®] Extrahera[™]. The Extrahera[™] is used to extract amphetamines (AMP), methamphetamine (MDA), 3,4-methylenedioxymethamphetamine (MDMA), and 3,4-methylenedioxy-N-ethylamphetamine, (MDEA) in comparison to manual vacuum manifold SPE followed by LC-MS/MS analysis.

Objectives: The intention of this study is to use the Biotage[®] Extrahera[™] automated solid phase extraction instrument and determine comparability of results with a validated manual extraction sample preparation methodology.

Methods: Twenty (n) presumptive positive amphetamine keratinized samples and standards were aliquoted twice. For the purpose of this study the keratinized matrix is n=11 human hair (head or body) samples and n=9 human nails (finger or toe).

Keratinized matrix was washed with acetone and powdered using stainless steel ball bearings in a mini bead beater instrument.

Four runs consisting of: Single Point Calibrator (100 pg/mg), 3 Calibration Verification Controls of a Low (40 pg/mg), Middle (125 pg/mg) and High (800 pg/mg), 1 negative control and 5 powdered keratinized donor samples were prepared. All controls and keratinized samples received buffer and internal standard (d11-Amphetamine, d14-d-Methamphetamine, d5-MDA, d5-MDMA and d5-MDEA). They were extracted through mixed mode SPE cartridges using the Extrahera[™] and a manual vacuum manifold.

Extrahera™ Parameters							
Step	Solvent	Pressure (Bar)	Time (sec- onds)				
Condition	MeOH	0.8	80				
	D.I. H2O	1	80				
Equilibrate	0.1M Phosphate Buffer	1	80				
Load	Premix samples 3x	0.5	120				
Wash 1	D.I. H2O	1	80				
Wash 2	1.0M Acetic Acid	1	80				
Wash 3	MeOH	0.8	80				
	Plate dry	5	60				
Elute	DCM/MeOH/NH4OH	0.5	120				
	Plate dry	5	60				

SPE procedure for both extractions were equivalent.

Both automated and manual samples were concentrated and analyzed using an identical validated LC-MS/MS method.

Results: Twelve donor keratinized samples confirmed positive values for AMP and MAMP analytes ranged from 137-19,501 pg/mg. Quantification values between identical samples extracted automatically and manually had a correlation coefficient of r=0.98 for AMP and r=0.99 for MAMP analytes.

All Calibration Verification controls were in acceptable criteria; less than +/- 10% of the Targeted Value for each analyte tested. The coefficient verification percent average compared to Targeted Value varied +/- 1.7-10.3% in Automated samples and +/- 0.6-5.2% in Manual samples.

Automated SPE instrument runtime averaged approximately 65 minutes and 7 minutes of set-up. Manual SPE procedures averaged 70 min.

Conclusion/Discussion: Automated SPE of amphetamines in keratinized matrix produced results comparable to previously validated manual SPE protocol in this study, with little to no method development and or troubleshooting. The automated calibrator and calibration verification samples met the standards of the laboratory and governing accreditation agencies such as CAP and ISO. Also, automated SPE method necessitated 90% less human interaction while producing equivalent results.

Outstanding analytical performance is built upon the foundation of sample preparation. The automated instrument's benefits are a catalyst to increase sample throughput, decrease errors, and most importantly gives the gift of time for chemists to focus on other aspects of the analytical process.

P46 - Solid phase extraction of natural cannabinoids and metabolites from urine and blood

Emily Eng*, Stephanie Reichardt, Abderrahim Abdelkaoui, Ritesh Pandya, UCT, Bristol, PA.

Background/Introduction: Natural cannabinoids are compounds found in the *Cannabis* plant. More than a hundred cannabinoids have been identified. Legally in the United States, marijuana is defined as "parts or products derived from the *Cannabis* plant with Δ^9 -Tetrahydrocannabinol (Δ^9 -THC) greater than 0.3% on a dry weight basis". Known for its psychoactive and euphoric effects, Δ^9 -THC has the highest levels in the subspecies *Cannabis Sativa*. As more states in the US legalize marijuana for medical and recreational use, it is important to be able to quantitate cannabinoids accurately and precisely from biological matrices.

Objectives: Develop an LC-MS/MS method that can separate isomers, Δ^9 and Δ^8 -THC. Develop and optimize extraction methods for cannabinoids from urine and blood with high recoveries and low matrix effects.

Methods: An LC-MS/MS method was developed on a Shimadzu Nexera LC-30AD with MS-8050. The analytes were separated on a SelectraCore[®] C18 UHPLC Column (100 mm x 2.1 mm, 2.7 μm). Mobile phase A was 0.1% formic acid in water and mobile phase B was 0.1% formic acid in methanol. Natural cannabinoids were extracted from urine and blood using Styre Screen[®] HLB (SSHLB) and Clean Screen[®] THC (CSTHC) solid phase extraction (SPE) cartridges respectively.

1 mL of urine was prepared by adding 1mL of acetonitrile (ACN) and 1 mL of phosphate buffer (pH 7, 0.1 M) for dilution and pH adjustment. This was followed by vortexing and centrifuging the samples for 10 minutes. SSHLB cartridges were conditioned with 2 mL of methanol and 2 mL of phosphate buffer. Samples were loaded onto the SPE cartridges before being washed with 3 mL of deionized water and 3 mL of 50% methanol (MeOH). After drying the columns, analytes were eluted using 3 mL of 60:40 methanol:hexane.

Blood samples were prepared using protein precipitation. To 0.5 mL of blood, 2 mL of cold ACN:acetone (75:25) was added before samples were vortexed and centrifuged for 10 minutes. The organic layer was decanted into 3 mL of phosphate buffer. CSTHC cartridges were conditioned with 2 mL methanol and 2 mL phosphate buffer. Samples were loaded onto the SPE cartridges before being washed with two aliquots of 3 mL of deionized water and two aliquots of 3 mL of 40% methanol. After drying the columns, analytes were eluted with 3 mL of 89:9:2 ACN:MeOH:acetic acid.

Results: A short 12-minute method was developed using UCT's SelectraCore[®] C18 column capable of separating isomers Δ^9 and Δ^8 -THC. Extraction methods were developed using Styre Screen[®] HLB for urine and Clean Screen[®] THC for blood. Six cannabinoids were extracted at three different concentrations (5, 25, and 50 ng/mL) from each matrix. Recovery of analytes from urine ranged from 93-103% (n=5) with relative standard deviation (RSD) <10%. Matrix effects for urine were low within ±25%. Recovery of analytes from blood ranged from 74-89% (n=5) with RSD values <5%. Matrix effects for blood samples were within ±26%.

Conclusion/Discussion: Two different SPE methods have been optimized for the analysis of cannabinoids from urine and blood. The addition of 1 mL of ACN during sample preparation of the urine extraction was optimized to prevent analytes from sticking to the sample test tube and loads more the analyte onto the SPE cartridge. This results in high recoveries. For the blood extraction method, the protein precipitation was optimized. ACN:acetone (75:25) resulted in the best comprise of high recoveries and lower matrix effects compared to other solvents such as ACN and ACN with 0.1% formic acid.

P47 - Analysis of drug-infused papers by ASAP-MS

Emily Lee* and Michelle Wood. Toxicology R&D, Waters Corporation, Cheshire, UK.

Background/Introduction: Drug misuse within UK prisons is prevalent and a major concern; it contributes to increased levels of aggression and violence amongst inmates, which put a strain on already-stretched resources, affecting the stability, security and overall effectiveness of the penal system. While traditional drug substances such as opiates, cocaine and cannabis continue to be widespread in prisons, the emergence of potent novel psychoactive substances (NPS) has significantly exacerbated the issue. Estimates of the use of NPS by up to 50% of inmates have been reported. Recently paper and other materials infused with drugs have been smuggled into UK prisons: including letters to inmates impregnated with drugs including NPS such as etizolam and synthetic cannabinoid receptor agonists. Reducing drug access is a key consideration in the overall strategy to reduce drug use in prisons by tackling supply and demand. Testing materials, received by inmates, may assist in this process.

Objectives: To assess the potential of RADIAN[™] ASAP Mass Detector, a compact device based on Atmospheric Solids Analysis Probe-Mass Spectrometry (ASAP-MS), as a simple, yet rapid, screening tool for drug infused paper.

Methods: Blank paper samples (80gsm) were treated with a range of 11 common illicit drug substances by one of two methods, pipetting or soaking. For the pipetting method, a 50µL aliquot of reference material (0.2 mg/mL and 1mg/mL in methanol) was added to 1x1cm pre-cut squares. For the soaking method, 4x4 cm sections of paper were placed into a beaker with diluted reference material. After infusion, samples were dried by placing flat on a glass tile for 30 min. Drug-free papers were prepared by the same methods using methanol in place of reference material.

Pre-cut paper samples or hole-punched samples from the soaked papers were extracted by sonication in 500µL methanol. A glass capillary was dipped into the extract supernatant for 10 sec, before loading into the ASAP device. Mass detection was performed using full scan MS *m/z* 60-650. To further enhance specificity, analysis was acquired simultaneously at four differing cone voltages. Data was processed by LiveID[™] software which provided real-time matching of acquired data to a spectral library and calculated an average match factor. A match factor of 800 (from max. 1000) was used as the minimum reporting criteria for a positive detection.

Results: ASAP-MS provides a direct analysis technique yielding mass spectrometry data without chromatographic separation. For all substances evaluated in this study, ionisation resulted in protonation (M+H⁺) of the analyte; ramped cone voltages of 15, 25, 35 and 50V, led to the generation of characteristic product ions which improved accuracy of drug identification.

Extraction times of 5, 10 and 15 min were evaluated for paper samples previously treated with drugs including cocaine, ketamine, MDMA, diazepam, THC, isotodesnitazene, 5F-MDMB-PINACA. Drugs were correctly identified with mean match factors ranging from 816 to 998. The increased sonication time showed no significant difference in response or match factor. Blank (methanol-only treated) papers screened negative for any drug substances. The 5 min extraction time was demonstrated to be suitable for differing paper types/thicknesses (*e.g.*, newspaper, greeting card, envelope, "glossy" magazine). For ease, and consistency of sampling, the use of a hole-punch (6 mm diameter) was also evaluated, match factors were lower but still exceeded 800. Responses were also lower reflecting the smaller area sampled. Data for anonymized authentic papers are presented.

Conclusion/Discussion: ASAP-MS appears promising as a rapid (<2 min) and simple screen for illicit drugs in paper samples and may be an effective tool to reduce access to drugs in prisons.

RADIAN[™] ASAP and LiveID[™] are trademarks of Waters Corporation.

© 2022 Waters Corporation
P48 - Quantitation of urine buprenorphine, norbuprenorphine, and naloxone on an automated platform using room temperature enzymatic hydrolysis and solid phase extraction

Dominic Minasi-Tapia, Tara Arends, **Brent Dawson*** and Ihuoma Igwilo. Redwood Toxicology Laboratory, Abbott, Santa Rosa, CA.

Background/Introduction: Buprenorphine, a semi-synthetic opioid, possesses partial agonist activity at the μ opioid receptor and antagonist activity at the κ receptor. Buprenorphine is primarily metabolized to an active metabolite, norbuprenorphine via N-dealkylation by CYP 3A4. Norbuprenorphine itself is a weak opiate agonist that has been reported to have a potency of a quarter of buprenorphine. Naloxone, μ receptor antagonist, is used to reverse the effects of opiate overdose.

The laboratory developed and validated an automated sample preparation method to simultaneously quantitate buprenorphine, norbuprenorphine and naloxone in human urine utilizing room temperature hydrolysis in 15 minutes. The method was validated in accordance with ANSI/ASB Standard 036 and has been utilized to analyze over 20,000 specimens from court-ordered drug monitoring and drug rehabilitation programs.

Objectives: To develop an automated sample prep LCMSMS method utilizing room temperature enzyme and solid phase extraction, for the analysis of Buprenorphine, norbuprenorphine and naloxone in urine. The attendee will be made aware of the benefits of using laboratory automation and room temperature hydrolysis on buprenorphine urine specimens. Additionally, a snapshot of the positivity rate and relative buprenorphine/norbuprenorphine ratios observed in urine specimens from court ordered drug monitoring and drug rehabilitation programs, will be discussed

Methods: Method validation studies included accuracy, precision, LOD/LLOQ, ULOL, carryover, matrix effects, stability, and interference from over 100 related and non-related drugs. Specimen preparation involved solid phase extraction (SPE) (Strata-XC 96 well format) clean-up of 0.5 mL of sample, post room temperature enzymatic hydrolysis (IMCS RT enzyme). The Hamilton[®] Nimbus platforms with the MPE² unit handled the sample aliquot, hydrolysis and SPE. Analysis was performed on a Prominence Liquid Chromatograph (Shimadzu) coupled to a 4000 QTrap mass spectrometer (SCIEX) by injecting 20 μ L on to a Raptor Biphenyl, 2.7 μ m, 50 x 4.6mm column. Mobile phases constituted 0.1% formic acid with 2 mM ammonium formate in deionized water and 0.1% formic acid with 2 mM ammonium formate in acetonitrile. Data are acquired within a 1.9-minute window with a total run time of 7.1 mins

Results: The LLOD and LOQ were 0.5 ng/mL and the ULOL was 1000 ng/mL, giving an $R \ge 0.9900$. Inter and Intra-day precision were below 4% with accuracies between 80-120%. No carryover was observed for concentrations up to 1,000 ng/mL for all three analytes. The %CV of the internal standard normalized matrix factors were <15%. No interference was observed from any of the over 100 related and non-related drugs tested. Recovery was determined to be >80% and Ionization enhancement/suppression were within ±25% for all analytes. Of 1372 samples analyzed in a typical week, positivity rates of 90.2%,90.8% & 73.5% were observed for Buprenorphine, Norbuprenorphine and naloxone respectively. An average ratio of buprenorphine to norbuprenorphine was observed to be 1:3.

In direct comparison with an established protocol utilizing the *Helix pomatia* β -glucuronidase, the experimental method showed acceptable agreement (within ±20%) for 81 samples analyzed. Proficiency samples tested also showed acceptable agreement with published ranges.

Conclusion/Discussion: An automated SPE method for the simultaneous analysis of Buprenorphine, Norbuprenorphine and Naloxone in urine has been developed in our laboratory utilizing room temperature hydrolysis. Reliable detection and quantitation were validated over a linear range from 0.5 – 1,000 ng/mL. Positivity rates and average analyte ratios of specimens extracted by this method in a typical week have also been reported.

P49 - Direct analysis of drug analytes in complex biological matrices using a molecularly imprinted polymer

Haley A Mulder^{1*}, Justin L Poklis², Adam C Pearcy¹, Matthew S Halquist¹ Departments of ¹Pharmaceutics, ²Pharmacology & Toxicology, Virginia Commonwealth University, Richmond, VA.

Background/Introduction: Sample preparation is a critical step in toxicological analyses. Current methods can be time consuming, expensive, and ultimately may lack specificity. These issues lead to the co-extraction of biological matrices that interfere with the instrumental analysis. Molecularly imprinted polymers (MIPs) are synthetic polymers with specific recognition sites for target analytes. MIPs are capable of selectively extracting analytes from complex biological matrices, creating better detection capabilities on analytical instruments. MIPs can be used in place of traditional high performance liquid chromatographic (HPLC) stationary phases, allowing for the direct analysis of samples.

Objectives: To create a MIP-based HPLC column for the direct analysis of drugs in biological matrices coupled with liquid chromatography tandem mass spectrometry (LC-MS/MS) systems without the need for sample preparation prior to instrumental analysis. This was first achieved by using a commercial polymer as a proof-of-concept, before engineering an in-house polymer specific for cotinine.

Methods: A commercial MIP specific for Tobacco Specific Nitrosamines (TSNAs) was obtained from Biotage (Uppsala, Sweden). The MIP analytical columns were . The analytical columns were prepared using a Teledyne (Thousand Oaks, CA) constant pressure packing system, and three MIP-HPLC columns were prepared. Direct analysis of NNN (N-nitrosonornicotine), NNK (4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone in nicotine products was performed using a SCIEX ExionLC 2.0 Binary Pump UPLC attached to a SCIEX SelexION 6500+ Q-Trap. The Mobile phases were 10 mM ammonium acetate, pH 5.5 and 0.1% formic acid in methanol, operated under gradient conditions for 5 minutes. The mass spectrometer was operated in multiple reaction monitoring mode using the following ions: NNN, 178 > 148 m/z; NNK, 208 > 122 m/z; and NNN-d4, 182 > 124 m/z and 182 > 152 m/z. The method was validated following the US Department of Health and Human Services Center for Tobacco Products Guidance for Industry. Column uniformity was assessed for retention time, peak area, calculated concentration, accuracy, asymmetry, tailing factor, and theoretical plate number (N).

An in-house polymer for cotinine using nicotine as the template was created through precipitation polymerization. The polymer's physical characteristics were determined by scanning electron microscopy. Cotinine in a urine matrix at 10, 100, and 1000 ng/mL were loaded onto the polymer. The polymer was washed with 10 mM ammonium acetate, pH 5.5 and heptane. Cotinine was eluted from the polymer with two fractions of methanol. The recovery of urine in the presence of other nicotine metabolites was also determined.

Results: The TSNA MIP-HPLC column method was successfully validated. Twenty-six nicotine and tobacco products contained TSNA concentrations, and were consistent with reported literature values. All parameters with the exception of theoretical plate number were within the accepted criteria (%RSD values < 15%). The theoretical plate numbers were determined to be between 200 and 237, owing to the large (50 μ m) commercial polymer. The in-house MIP polymer was < 10 μ m in size with a spherical morphology. Cotinine was successfully recovered in urine matrix on the in-house polymer (77-103% recovery), and the presence of analytes did not interfere with cotinine's recovery.

Conclusion/Discussion: The commercial TSNA-MIP HPLC column was effectively used for direct analysis of TSNAs in nicotine and tobacco products. The large polymer size of the commercial polymer created poor chromatographic resolution between analytes. The creation of the in-house polymer for cotinine, however, had < 10 μ m particle size. The in-house polymer was suitable for extractions of cotinine prior to instrumental analysis and can potentially be used for direct analysis in HPLC columns. The use of molecularly imprinted polymers can be further expanded for other drug analytes, such as drugs of abuse or biomarkers of exposure.

P50 - A chemical profile of commercially available kratom products in Richmond, Virginia

James H. Fleming, M.S.^{*1,2}, Carol M. Babyak, Ph.D.³, Emanuele A. Alves, Ph.D.^{2,4} ¹Office of the Chief Medical Examiner, Washington DC, 20024. ²Department of Forensic Science, Virginia Commonwealth University, Richmond VA, 23284. ³A. R. Smith Department of Chemistry and Fermentation Sciences, Appalachian State University, Boone NC, 28608. ⁴National School of Public Health Sergio Arouca, Oswaldo Cruz Foundation, Rio de Janeiro, Brazil, 21041-210.

Background/Introduction: Kratom, also known as "Ketum" or "Biak-biak", is a novel psychoactive substance that has gained popularity in the United States. Kratom originates from the leaves of the *Mitragyna speciosa* tree, native to Southeast Asia. Kratom products are sold on the Internet, in tobacco shops, and in convenience stores. Mitragynine is the main psychoactive substance in kratom, followed by its derivative 7-hydroxymitragynine, which both play a key role in kratom central nervous system effects. Over 40 indole alkaloids have been identified within the plant. In excessive amounts, kratom leaves can cause stupor, sweating, dizziness, nausea, and dysphoria – effects that are usually expressed in opioid overdose. Besides its psychoactive properties, kratom products might contain detrimental elements such as heavy metals, which are absorbed by the plant from the soil during its cultivation, and during the grounding process to prepare the final product (powder or teas). The excessive consumption of heavy metals can lead to health disorders, such as damage to the kidneys and reproductive system, lung problems, bone damage, and even cancer.

Objectives: To develop a chemical profile of commercially available kratom products in Richmond, Virginia. This included the development of an inorganic profile for the quantitation of heavy metals using Inductively Coupled Plasma-Optical Emission Spectroscopy (ICP-OES). A qualitative organic profile was obtained using Direct Analysis in Real Time-Mass Spectrometry (DART-MS). Kratom alkaloids were confirmed with Gas Chromatography-Mass Spectrometry (GC-MS).

Methods: Twenty-nine kratom products were purchased from local smoke and tobacco shops in Richmond, Virginia including 9 powders, 2 capsules, 5 teas, 12 extracts/isolates, and 1 carbonated beverage. Powder samples underwent an acid digestion prior to analysis by an Agilent 5110 ICP-OES in radial mode. Elements included Al, As, Cu, Fe, Mg, Mn, Ni, and Pb. For the organic profile, a JOEL JMS T100LC Accu-ToF DART-MS was used and a capillary tube was dipped in a methanolic solution obtained from kratom samples and introduced into the helium stream of the DART-MS. DART-MS results were confirmed with a 10 minute GC-MS method and reference standards.

Results: Three kratom tea samples were found to have manganese levels above the tolerable upper intake level of 11 mg/ day. The tolerable upper intake level is the highest amount of a substance that can be consumed on a daily basis with no adverse effects. Manganese levels for these samples ranged from 56.1-284.7 mg/serving. Overexposure to manganese can lead to Manganism, which exhibits Parkinsonian symptoms including dystonia, facial muscle spasms, and body tremors. Kratom alkaloids were present and able to be seen with DART-MS. However, DART was not able to separate mitragynine diastereomers, including speciogynine, paynantheine, and speciociliatine. Diastereomers were able to be separated with GC-MS.

Conclusion/Discussion: This work highlights the need for proper regulation not only within the kratom industry, but for all products that are sold in smoke and tobacco shops. Manufacturers should be held accountable and be required to list all ingredients on the label. Finally, manganese should be included in the panel of metals tested for in quality assurance of kratom products, if any, as it poses a risk to users.

P51 - The discovery of phenibut in a commercially available product: a case study

James H. Fleming, M.S.^{*1,2}, Justin L. Poklis, B.S.³, Michelle R. Peace, Ph.D.², Emanuele A. Alves, Ph.D.^{2,4} ¹Office of the Chief Medical Examiner, Washington DC, 20024

²Department of Forensic Science, Virginia Commonwealth University, Richmond VA, 23284.³Department of Pharmacology & Toxicology, Virginia Commonwealth University, Richmond VA, 23284.⁴National School of Public Health Sergio Arouca, Oswaldo Cruz Foundation, Rio de Janeiro, Brazil, 21041-210.

Background/Introduction: Phenibut is an anxiolytic and nootropic substance that was first utilized in Russia in the 1960's. Phenibut is GABA-mimetic, primarily as a GABA-B receptor agonist with minimal activity at the GABA-A receptor. Phenibut is readily available on the Internet and is not a controlled substance under the Controlled Substances Act in the United States. Adverse effects of phenibut include tremors, decreased appetite, agitation and insomnia. In this study, a sample was obtained from a tobacco shop in Richmond, Virginia. The product was labeled as "Moon Water" and contained a yellow liquid. The Moon Water sample was labeled to promote energy and focus. Labeled ingredients included filtered water, fructose, citric acid, < 2% ascorbic acid, maltodextrin, sodium acid pyrophosphate, sodium citrate, magnesium oxide, calcium fumarate, artificial color, and tocopherol. However, phenibut was present in the sample without any information for the consumer.

Objectives: The objective was to screen the product using Direct Analysis in Real Time-Mass Spectrometry (DART-MS) and Gas Chromatography-Mass Spectrometry (GC-MS) for the detection of active compounds not described in the label.

Methods: A JOEL JMS T100LC Accu-ToF DART-MS was used, and a capillary tube was dipped in a methanolic solution obtained from the sample and introduced into the helium stream of the DART-MS. The sample was subjected to a 1:5 dilution in methanol for GC-MS analysis. A Shimadzu QP-2020 GC-MS was used with an initial screening method. A final confirmation method included an initial oven temperature of 100°C, ramping at 15°C/minute until 300°C and a final hold of 6 minutes for a total run time of 10.44 minutes.

Results: DART-MS showed the presence of labeled ingredients, as well as caffeine, which was not on the label. GC-MS showed the presence of phenibut. Phenibut exhibits agonism at the GABA receptors, specifically the GABA_B receptor. High doses of phenibut can result in combativeness, delirium, and aggression. Phenibut usage can result in dependence and subsequent withdrawal, with effects ranging from anger, irritability, insomnia, decreased appetite, and heart palpitations.

Conclusion/Discussion: This work highlights the need for proper regulation of commercially available products, specifically those sold in smoke and tobacco shops. The presence of psychoactive substances that are not labeled on the product are a public health concern and an upmost risk to the consumer.

P52 - Consolidating LC-MS/MS method conditions for the analysis of alcohol metabolites, barbiturates, and drugs of abuse in urine

Jamie York*. Restek Corporation, Bellefonte, PA. .

Background/Introduction: To simplify the analysis of alcohol metabolites, barbiturates, and drugs of abuse in urine, three different methods were developed for each analyte class using the same analytical column and mobile phase compositions. Consolidating LC-MS/MS method conditions allows labs to streamline their analytical testing processes and reduce overall costs.

Objectives: The primary objective is to demonstrate the analysis of alcohol metabolites and ESI+/ESI- therapeutic drugs, drugs of abuse, and their metabolites in urine using one set of LC-MS/MS method conditions. Methods: A panel of 136 ESI+/ESI- therapeutic drugs, drugs of abuse, and their metabolites, as well as biomarkers of alcohol consumption, were all analyzed using a Force Biphenyl 50 x 3 mm, 2.7 µm analytical column equipped with a Force Biphenyl EXP guard column cartridge and a 0.2 µm Ultra Shield frit. Mobile phase A consisted of 0.1% formic acid in water and mobile phase B consisted of 0.1% formic acid in methanol. All methods utilized a column oven temperature of 30 °C. The positive mode isobars utilized gradient conditions with a total cycle time of 10 minutes. Urine samples underwent hydrolysis to convert glucuronide conjugates to their free forms. Barbiturates, THCA-A, and THC-COOH were analyzed in ESI- mode by gradient conditions and had a total run time of 5 minutes. These compounds were spiked into urine and diluted 10-fold with water. Finally, alcohol metabolites were monitored in ESI- with a total analysis time of 5 minutes. Samples were prepared by diluting with water 10-fold and injecting 10 µL.

Results: The Biphenyl stationary phase has unique selectivity due to the pi-pi interactions that occur between the phase and most drugs and drug metabolites when compared to a routine C18 phase allowing for improved resolution of isobars. A demonstration of the powerful selectivity of this methodology is exemplified for seven isobaric compounds sharing the m/z 286. These compounds include morphine, hydromorphone, norcodeine, norhydrocodone, 7-aminoclonazepam, pentazocine, and asenapine, which are all baseline resolved. Urinary interferences that are particularly problematic in alcohol metabolite testing were resolved without the use of buffer or additional mobile phases helping to streamline analytical testing processes. The ESI- panel that includes barbiturates is able to achieve partial resolution of amobarbital and pentobarbital which allows labs to identify which barbiturate is present in their sample, which may eliminate the need for confirmatory testing.

Conclusion/Discussion: A panel of 136 ESI+/ESI- therapeutic drugs, drugs of abuse, and their metabolites, as well as biomarkers of alcohol consumption, were all analyzed using the same column and mobile phases. This work demonstrates that one LC-MS/MS setup is possible for the analysis of multiple panels. This allows the user to simplify testing procedures, save time, and ultimately reduce costs.

P53 - A one year review of the application of a LC-MS/MS screening method for suspected blood DUI cases

Jazmine Adams*, Kristin Kahl, and Lisa Reidy. University of Miami, Miller School of Medicine, Toxicology Laboratory.

Background/Introduction: Methodologies used to screen biological specimens (i.e., blood and urine) for suspected driving while under the influence (DUI) and/or driving while intoxicated/impaired (DWI) cases can vary among toxicology laboratories. Some forensic laboratories use immunological-based testing (i.e., ELISA); however, there can be limitations regarding target analytes due to kit availability/sensitivity and the cost of reagents. Liquid chromatography-tandem mass spectrometry (LC-MS/MS) allows for streamlined and cost-effective testing with improved sensitivity and flexibility concerning target analytes. This presentation will discuss blood specimen results and drug trends for suspected DUI cases obtained by LC-MS/MS screening methodology.

Objectives: The objective of this study is to conduct a review of the application of a LC-MS/MS screening method for the detection and frequency of compounds in common drug classes in suspected blood DUI case specimens submitted to the University of Miami Toxicology Laboratory (UMTL).

Methods: Blood specimens in suspected DUI cases submitted to the UMTL between July 2021 and May 2022 were analyzed for this review. Blood specimens were initially analyzed for ethanol using headspace-gas chromatography-flame ionization detector/mass spectrometry (HS-GC-FID/ HS-GC-MS/FID). Blood specimens with alcohol concentrations less than 0.150 g/100 mL were reflexed for additional testing using the LC-MS/MS methodology per laboratory protocol.

The 82-drug targeted LC-MS/MS blood method utilizes 200 μ L of blood fortified with an internal standard (IS) solution containing 37 isotopically labeled compounds. Samples underwent protein precipitation with cold acetonitrile containing 0.1% formic acid, centrifugation, and sample clean-up with Phenomenex Phree Phospholipid removal columns. Sample extracts were evaporated using nitrogen and reconstituted with mobile phase. Sample extracts were injected onto an Agilent Poroshell 120 EC-18 3 x 50 mm, 2.7 μ m column. Gradient-elution chromatographic separation was achieved using an Agilent 1260 HPLC system coupled to an Agilent 6460 MS/MS in positive, dynamic multiple reaction monitoring (dMRM) mode for identification.

The LC-MS/MS method was validated, in accordance with the ANSI/ASB Standard 036, and included studies that determined the limits of detection (LODs), matrix effects, and possible interferences for the targeted drugs.

LC-MS/MS results for suspected blood DUI cases were evaluated to determine the prevalence of drug classes within the sample population.

Results: A total of 202 suspected DUI cases with blood specimens submitted to the UMTL were reviewed; 80 cases did not require additional testing due to ethanol results exceeding 0.150 g/100 mL. Out of the 122 blood suspected DUI specimens screened using the LC-MS/MS methodology, 76% had positive results for one or more compound(s) within the following drug categories: amphetamines, benzodiazepines, cannabinoids, opioids, and other. Drugs categorized as "other" consists of the following classes: antidepressants, antipsychotics, and anticonvulsants. A total of 15, 22, 52, 28 and 21 cases had compounds detected in the amphetamines, benzodiazepines, cannabinoids, opioids, and other drug groups, respectively. A total of 27 cases had no compounds detected. Out of the 93 positive cases, 80% had all compound(s) confirmed by a secondary test. There was 16, 11, and 8 cases that had compounds detected in the following drug class combinations: cocaine with opioids, cannabinoids with cocaine, and benzodiazepines with cocaine and opioids, respectively.

Conclusion/Discussion: Although the LC-MS/MS screening methodology was not utilized for all blood specimens in suspected DUI cases submitted to the UMTL, this review provides insight regarding the frequency of positively identified compounds within major drug categories as well as multi-drug use trends. LC-MS/MS methodology allows for the addition of emerging drugs while being more cost-effective and conducive to laboratory turnaround time via decreasing delays associated with immunological assay development.

P54 - Analysis of benzodiazepines using liquid-chromatography mass spectrometry-mass spectrometry.

Jonathan T. Tomko^{*1} M.S.F.S , Alison L. Goetz,¹ B.S. , Amy L. Rutgers¹ M.S , Leah J. Macans¹ B.S. ¹Wisconsin Department of Justice-Division of Forensic Sciences (DFS)- Milwaukee, WI.

Background/Introduction: Novel benzodiazepines are prevalent within the community and are used to circumvent prosecution under the Controlled Substances Act. However, it has been demonstrated that benzodiazepines can cause impairment and thus affect the ability to safely operate any vehicle on roadways. At DFS, only one novel benzodiazepine is within the current scope (etizolam). Currently, DFS's procedure includes hydrolysis and solid phase extraction on day one which takes up to six hours to complete. These underivatized samples are analyzed by GC-MS for five benzodiazepines (diazepam, 7-amino-flunitrazepam, alprazolam, etizolam and midazolam). On day two, samples are derivatized for one hour and analyzed for the remaining eight benzodiazepines (7 amino-clonazepam, hydroxy alprazolam, hydroxy triazolam, nordiazepam, oxazepam, temazepam, hydroxy ethyl-flurazepam, and lorazepam). At the minimum, two and half days are required to complete analysis before the data can be reviewed.

Objectives: The objective of this research was to shorten the analysis time, reduce consumption of case samples, expand the current scope of analysis, and move the assay from gas chromatography mass spectrometry (GC-MS) to liquid chromatography mass spectrometry-mass spectrometry (LC/MS/MS). Drug cases routinely screen positive using enzyme-linked immunoassay (ELISA) for the benzodiazepine drug class, but none detected is reported from the confirmation method. This is because the existing method lacks the ability to confirm many of these drugs.

Methods: Blood samples are extracted by solid-phase extraction using United Chemical Technologies Clean Screen DAU SPE columns. After 0.5 mL of blood is extracted, the samples are analyzed by electrospray ionization tandem mass spectrometry in positive ion mode (Waters ACQUITY UPLC H-class/Xevo TQD). An ACQUITY Ultra-Performance Liquid Chromatography BEH C18 column (2.1 mm*100 mm, 1.7 μm) is used for separation with the following gradient:

Time (mins)	%Water	%Methanol	2% formic acid
Initial	65	30	5
2.50	30	65	5
3.25	25	70	5
4.50	18	77	5
4.51	5	90	5
5.80	5	90	5
5.91	65	30	5
9.00	65	30	5

Results: This method was validated to ANSI/ASB standard 036, Standard Practices for Method Validation in Forensic Toxicology (1st edition, 2019). No interferences were observed from the matrix, internal standards, high drug concentrations and other commonly encountered drugs which include fentanyl, nor-fentanyl, codeine, hydrocodone, oxycodone, oxymorphone, morphine, hydromorphone, 6-monoacetyl morphine, amphetamine, methamphetamine, ephedrine, pseudoephedrine, MDA, MDMA, phentermine, tetrahydrocannabinol and metabolites, cocaine and metabolites. (List not all inclusive)

Flunitrazepam, 7-amino-flunitrazepam, midazolam, hydroxy etizolam, triazolam, hydroxy-triazolam, bromazepam, chlordiazepoxide, nor-chlordiazepoxide, clobazam, clonazolam, delorazepam, demoxepam, diclazepam, estazolam, flualprazolam, flubromazepam, flubromazolam, flurazepam, hydroxy ethyl-flurazepam, lormetazepam, nimetazepam, nitrazepam, phenazepam, pyrazolam, zaleplon, and zopiclone are qualitative only.

Drug	Quantitative range		Low	Mid	High
Drug	(ng/mL)	(ng/mL)	(% Bias)	(% Bias)	(% Bias)
7-amino clonazepam			6.11	4.90	1.01
Alprazolam			2.14	1.30	0.56
Clonazepam			3.34	3.47	1.60
Etizolam	5-240	5	2.30	1.52	7.11
Lorazepam			-0.93	-1.60	-3.62
Zolpidem			4.95	5.95	0.66
Hydroxy alprazolam			2.75	5.70	1.39
Diazepam		10	5.41	7.48	3.89
Nordiazepam	10,1000		13.60	13.37	5.18
Oxazepam	10-1000		8.27	7.39	4.97
Temazepam			3.94	3.42	3.27

Conclusion/Discussion: The SPE extraction for LCMSMS analysis and processing of 38 benzodiazepines was reduced to approximately 12 hours. This will allow for quicker results and more time for other processes. Currently, this new method is not approved for casework because dilution integrity has not been assessed and it will be qualitatively evaluated with urine samples. It is expected that benzodiazepine ELISA positive results will have more reportable drug confirmations once this method is approved because of an increased selectivity of this new method. In turn, this helps the community identify any drugged driving trends and district attorneys prosecute those that endanger others on Wisconsin roadways.

This research was supported by Wisconsin Department of Justice-Division of Forensic Sciences.

P55 - When party balloons became taboo: gas chromatograph carrier gas conversion from helium to hydrogen

Joseph H. Kahl*, George W. Hime, Diane M. Boland

Miami-Dade Medical Examiner Department Toxicology Laboratory, Miami, FL, USA.

Background/Introduction: Historically, helium has been the carrier gas of choice for many forensic laboratories that operate gas chromatographs (GC) to analyze their samples. Helium provides good chromatographic separation, is inert, and is safe to use; however, the global availability of helium has been in decline in recent years, and its cost has increased significantly. This leaves many forensic laboratories at greater risk of either running out of helium due to the global supply shortages or depleting their gas budgets due to its ever-increasing cost.

One alternative to helium as a carrier gas is converting existing GCs to utilize a different carrier gas: hydrogen. The use of hydrogen provides forensic laboratories with a multitude of benefits, including shorter chromatographic run times with comparable resolution, extended column life and reduced column bleed, a significant cost savings, and it can be readily generated from the electrolysis of water. The increased safety risks of using hydrogen as a carrier gas can be mitigated with the simple addition of a hydrogen safety sensor into the GC and the use of a low-volume, low-pressure hydrogen generator; this can prevent the possibility of a rapid leak from compressed hydrogen cylinders that could create an explosion hazard.

Objectives: The objective of this project was to convert a GC with dual flame ionization detectors (FID) to utilize hydrogen as a carrier gas and validate a method for the simultaneous identification and quantification of methanol, ethanol, isopropanol, and acetone in postmortem specimens.

Methods: A Shimadzu GC-2030 with dual FIDs and an AOC-6000 multi-injection rail system autosampler with headspace sampling capabilities was retrofitted with a hydrogen safety sensor and reconfigured to utilize hydrogen as the carrier gas. Both helium and hydrogen have similar separation efficiencies on capillary GC columns, albeit at much different linear velocities. Due to this, the previously validated method utilizing helium as the carrier gas to simultaneously identify and quantify methanol, ethanol, isopropanol, and acetone in postmortem specimens needed to be re-optimized to closely replicate the previous method with little to no chromatographic or detector response differences. This included changing the total flow rate of the carrier gas through the GC columns as well as the hydrogen:air flow ratio to the FIDs. Once the method was re-optimized, it was then re-validated following ANSI/ASB Standard 036, *Standard Practices for Method Validation in Forensic Toxicology*.

Results: The re-optimized method utilizing hydrogen as the carrier gas resulted in little to no retention time shifting of the target analytes or internal standard. Additionally, there were no observed changes in the background signal or detector signal responses for the target analytes and internal standard across their respective working calibration ranges. All four analytes exhibited a quadratic curve fit with a 1/x weighting and a working calibration range of 0.02-0.5 g/dL for methanol, ethanol, and isopropanol, and 0.002-0.05 g/dL for acetone. Bias was within $\pm 10\%$, precision was below 10%, and there were no known interferences on either GC column from abused and commonly encountered volatile organic compounds. The results of the method re-validation were equivalent to those of the previous method that utilized helium as the carrier gas.

Conclusion/Discussion: A previously validated method that utilized helium as a carrier gas to simultaneously identify and quantify four volatiles in postmortem specimens was re-optimized and re-validated utilizing hydrogen as the new carrier gas after a simple instrument reconfiguration. Consequently, the laboratory was able to reduce its reliance on outside vendors to supply compressed helium cylinders that are in high demand and low supply, as well as save money by using a cheap and renewable carrier gas to achieve comparable results.

P56 - Assessment of commercially available devices for the removal of histamine from red and white wines.

Judith Rodriguez Salas^{*1}, Francis X. Diamond¹, Barry K. Logan¹.

¹Center for Forensic Science Research and Education (CFSRE) at the Fredric Rieders Family Foundation, Willow Grove PA 19090.

Background/Introduction: Wine has been produced for thousands of years and it is one of the most commonly consumed alcoholic beverages around the globe. Histamine is produced in wine during fermentation, as naturally occurring histidine from the grapes is decarboxylated to histamine. In spite of its popularity, there is a portion of the population who do not tolerate histamine well and may encounter unpleasant side effects such as itchy watery eyes, rhinorrhea, headaches and flushing. There are many commercially available products claiming to remove the histamine while preserving the quality of the beverage taste and aroma. Directions are generally to swirl the device in a glass of wine or pour the wine through a pour spout to remove the histamine.

Objectives: The objective of this project was to optimize and validate a previously published quantitative method for the analysis of histamine in red and white wines after treatment with devices that claimed to remove the histamine. Analysis was performed by liquid chromatograph tandem mass spectrometer (LC-MS/MS) with a Hydrophilic Interaction Chromatography (HILIC) column well suited to the analysis of highly polar analytes. A selection of wines was tested before and after using a variety of commercially available histamine removal products to evaluate their claims of being able to remove up to 90% of histamine in wine.

Methods: Sample preparation consisted of simple dilution of aliquots of store-bought wines. Samples were filtered by passing them through a 0.2 μ m nylon filter using a 1 mL luer-lock syringe to apply pressure. Wine was filtered and a 100 μ L was aliquoted and diluted in 1000 μ L of mobile phase (MPA:MPB 60:40 with mobile phase A 0.1% formic acid in water and B 0.1% formic acid in acetonitrile). 50 μ L of a deuterated internal standard, histamine-D4, was added to all the samples at a concentration of 1 ng/ μ L prepared in 0.1% HCl. A seven-point calibration curve from 5 - 2000 ng/mL was prepared using histamine-free red grape juice (RGJ) and white grape juice (WGJ) as matrix surrogate. A fit-for-purpose validation for this application was designed using portions of the ANSI/American Standards Board (ASB) Standard 036, consisting of assessment of calibration model, bias and precision, limit of detection (LOD), limit of quantitation (LOQ), carryover, internal standard interference, ion suppression/enhancement and commonly encountered interferences in wine.

Analysis was conducted on an Agilent 1200 High Performance Liquid Chromatograph coupled to an Agilent 6430 Triple Quadrupole Mass Spectrometer (LC-MSMS). The mode selected for liquid chromatography was HILIC. The mass spectrometer was operated in positive ion electrospray using MRM. Quantitation was achieved using a Cogent Diamond Hydride 100A (4 μ m 150 x 2.1mm) analytical column at a temperature of 40°C. A total of three red wines, and three white wines will be analyzed using different commercially available histamine removal devices placed in 200 mL of the wine for 3, 5 or 30 minutes.

Results: All validation requirements were met. The LOD and LOQ were determined to be 5 ng/mL.

The histamine content of the untreated red wines was between 3300 ng/mL and 4600 ng/mL. Preliminary results using one of the testing devices, that was placed in the wine for 5 or 30 minutes, has shown that at 5 minutes, there was a minimal reduction of histamine (<1%), and after 30 minutes between 8 and 10% of the histamine had been removed.

Conclusion/Discussion Histamine in wine is well-documented to have an adverse effect on individuals with histamine intolerance. The commercial products evaluated in this assessment only had a minimal impact on the removal of histamine and are unlikely to counteract the adverse effects in histamine sensitive individuals. Testing of other devices is ongoing.

P57 - Comparison of LC-QqQ-MS and DART-QqQ-MS for the detection of drugs in authentic hair specimens.

Kaylyn Keith, B.S.*, Anthony P. DeCaprio, Ph.D., Anamary Tarifa, Ph.D., Florida International University, International Forensic Science Research Institute, Miami, FL 33199.

Background/Introduction: Drug testing in hair is an attractive alternative analysis in forensic toxicology due to its non-invasive nature, better stability of drugs in the matrix and simple specimen collection. For cases of Drug Facilitated Crime (DFC), hair analysis is extremely useful, especially if the crime is reported past the typical drug detection window in blood or urine (5 days). The hair matrix offers a wider detection window of weeks to months after ingestion. A major challenge in forensic hair analysis is the detection of single doses of drugs in DFC cases. Analyzing single dose exposures requires high sensitivity and selectivity analysis methods.

Often, forensic hair analysis is conducted by liquid chromatography (LC) coupled to triple quadrupole mass spectrometry (QqQ-MS). This is a well-developed technique that allows screening, confirmation, and quantitation of drugs; however, sample preparation is very time-consuming. An alternative technique may be Direct Analysis in Real Time mass spectrometry (DART-MS). DART-MS has the ability to analyze compounds using ambient ionization for a more rapid detection of drugs and without extensive sample preparation.

Objectives: For this study, a DART-QqQ-MS screening method was developed and a previously developed (in the PI laboratory) LC-QqQ-MS screening method was optimized for drugs in hair. The DART-QqQ-MS and LC-QqQ-MS methods were compared for analysis time, sensitivity, and selectivity capabilities. Both analytical techniques were evaluated using authentic Hair Reference Material (HRM) obtained from chronic drug users (RTI International).

Methods: A total of 40 drugs relevant to DFC cases were included in a targeted LC-QqQ-MS method in dynamic MRM mode, including 40 corresponding internal standards. Sample preparation for analysis of HRM consisted of weighing, decontamination and drying, pulverization, solvent extraction, and extract filtering. Decontamination consisted of 30-min washes 1X with water and 3X with DCM. The solvent extraction consisted of 250 μ L of methanol, acetonitrile, and 2 mM ammonium formate (25:25:50, v/v, pH 5.3) and incubating with shaking at 37°C for 2 h. An Agilent 1290 Infinity UHPLC coupled to a 6470 QqQ MS was utilized in this work with a 1.8 μ m Zorbax Eclipse Plus C18 analytical column. The DART SVP source (IonSense, Saugus, MA) used helium as the desorption/ionization gas.

Results: The 40 drugs tested were chromatographically separated or contained different MRM transitions to allow quantitation and identification of each compound. The LC-QqQ-MS method provided an LOD and LOQ range of 0.04-2 ppb (pg/ mg) and 0.1-7 ppb, respectively, for all compounds. The calibration curves showed linearity with R² values in the range of 0.9976-0.9998, except for lorazepam (R²= 0.9913). The proposed decontamination, homogenization and extraction methods were evaluated using HRM. Concentration of drugs in HRM extracts were calculated using the equation of the line from the calibration curves. All compounds present in the HRM were detected at the indicated concentration by LC-QqQ-MS. The DART-QqQ-MS method showed a 100 ppb LOD for 11 compounds, demonstrating applicability as a screening method.

Conclusion/Discussion: The sample preparation presented here provides sufficient recovery for the detection of drugs in HRM by either MS method. The LC-QqQ-MS method demonstrated good selectivity and sensitivity for the selected compounds, however, DART-QqQ-MS analysis could prove to be a faster method for identification of drugs in hair. Future work will involve the applicability of the methods described herein for the analysis of a single dose of drugs in hair.

P58 - Sample preparation methods for the analysis of cannabis exposure biomarkers in exhaled breath condensate and oral fluid.

Meena Swaminathan, M.S.*, Anamary Tarifa, Ph.D., Anthony P. DeCaprio, Ph.D., Florida International University, International Forensic Science Research Institute, Miami, FL 33199.

Background/Introduction: The increase in cannabis consumption for its perceived medical benefits and recreational utilization has led many U.S. states to legalize cannabis products. There is a current challenge in forensic toxicology to identify biomarkers of cannabis exposure, other than the commonly detected delta-9-tetrahydrocannabinol (Δ^9 -THC) and its metabolites, with simple sample collection procedures to evaluate cannabis consumption patterns.

Exhaled breath condensate (EBC) and oral fluid (OF) are alternative, non-invasive matrices that hold promise for presence and possible detection of cannabis exposure. EBC consists of condensed water vapor and volatile and non-volatile compounds. Potential advantages of EBC as a matrix include ease of collection and presence of a wide variety of analytes, including markers of respiratory disease, metabolites, and drug compounds. EBC is mainly used in clinical settings and the presence of drugs has been detected, however, there are no reports on cannabinoid detection. OF is currently being explored as a matrix for cannabis exposure analysis. The current sample preparation methods for OF and EBC include the use of protein precipitation, solid phase extraction (SPE), and lyophilization for EBC. However, these approaches require rigorous method development, are time consuming, and utilize instrumentation that may not be widely available.

Objectives: This work focuses on developing simple and fast sample preparation methods to quantify cannabinoids and metabolites as potential cannabis exposure biomarkers in EBC and OF using a previously optimized liquid chromatography triple quadrupole mass spectrometer (LC-QqQ-MS) method in dynamic multiple reaction monitoring (dMRM) mode. The developed and optimized methods will be employed in the analysis of authentic specimens from cannabis smokers and non-smokers.

Methods: A total of 25 cannabinoids and metabolites were targeted for analysis, including six internal standards. An Agilent 1290 UHPLC coupled to a 6470 LC-QqQ-MS with electrospray ionization in positive mode was utilized for this work. Chromatographic separation used a Zorbax 120 EC-C18 column (3.0 x 100 mm, 1.8 μm) and a step gradient. OF was collected using Quantisal[™] (Immunalysis) and EBC was collected using RTube[™] (Respiratory Research) devices. After collection of OF, the pads were placed in either the provided buffer, 3 mL methanol, or 3 mL cold (-20°C) acetonitrile (ACN) to evaluate which solvent yielded higher recoveries and reproducibility. Prior to sample preparation, the pads were removed from the buffer or solvent and the solution was filtered. For OF, direct injection, evaporation/reconstitution, and protein precipitation were tested. For evaporation/reconstitution, a 1 mL aliquot, then evaporated to dryness and reconstituted in ACN. For protein precipitation, cold ACN was added to a 1 mL aliquot, then evaporated to dryness and reconstituted in ACN. For EBC, evaporation/reconstitution and protein precipitation were tested as described previously with a 500 µL aliquot.

Results: For OF, extraction with ACN followed by evaporation/reconstitution yielded best recoveries and reproducibility. The recoveries ranged from 34-121%, with most >70%, except for cannabigerol monoethyl ether (34%), cannabichromene (48%), and cannabicyclol, cannabinol, and 11-nor-cannabinol-9-carboxy-acid (~60%). For EBC, the mean recoveries were not significantly different for the two sample preparation methods. Recoveries for acidic cannabinoids and metabolites were substantially higher (80-90%) than for the neutral cannabinoids (<10%).

Conclusion/Discussion: Simple, fast, and reliable sample preparation methods were developed for acidic cannabinoids and metabolites in OF and EBC. Additional work is being conducted to optimize extraction of neutral cannabinoids from these matrices. These methods will be applied in the detection of cannabis exposure biomarkers in ongoing research involving OF and EBC samples obtained from a human cohort of medicinal and recreational cannabis smokers with different user profiles.

P59 - Simultaneous analysis of free steroids and sulfate conjugates by solid-phase extraction and LC/MS/MS

Stephanie Reichardt*, Emily Eng, Abderrahim Abdelkaoui, Ritesh Pandya, UCT, Bristol, PA.

Background/Introduction: Steroid analysis in urine involves monitoring glucuronide conjugates and requires an enzyme hydrolysis step to cleave glucuronic acid, resulting in improved detectability of the free form. However, recently, there is growing interest in the direct analysis of sulfate conjugates due to a couple of factors. First, the ratio of glucuronide metabolites to sulfate metabolites varies from person to person, but the enzymes used for hydrolysis have little or no sulfatase activity. The ratio of the conjugates is also time-dependent since sulfates are excreted at a slower rate. Second, current research suggests that the analysis of steroid sulfates can potentially increase the detection window for steroid abuse. This poster outlines a method for the concurrent extraction and analysis of a total of sixteen free and sulfated steroids from human blood and urine using reverse-phase solid-phase extraction (SPE) and LC-MS/MS.

Objectives: Develop an LC-MS/MS and solid phase extraction method for the simultaneous analysis of free steroids and sulfated conjugates.

Methods: UCT's Styre Screen[®] HLB cartridges were utilized for extraction. Samples were prepared at a concentration of 25 ng/mL from a stock solution of free and sulfated steroid standard mix. Urine samples were prepared by diluting 0.5 mL of sample in 1.5 mL of phosphate buffer (pH 7.0, 0.1M). HLB cartridges were conditioned with 3 mL of methanol (MeOH) and equilibrated with 3 mL of phosphate buffer. The samples were loaded onto the cartridge and washed with 3 mL of 60 mM HCl followed by 3 mL of 30% MeOH. The target analytes were eluted with 3 mL of MeOH.

Blood samples were prepared by diluting 0.5 mL of sample in 1.5 mL of acetonitrile (ACN), mixing, and centrifuging. The supernatant was evaporated under a gentle stream of nitrogen at 40°C and reconstituted in 2 mL of phosphate buffer. HLB cartridges were conditioned with 3 mL of MeOH and equilibrated with 3 mL of phosphate buffer. The samples were loaded onto the cartridge and washed twice with 3 mL of 60 mM HCl and twice with 3 mL of 30% MeOH. The target analytes were eluted with 3 mL of 50:50 MeOH:ACN. After evaporating, the extracts were reconstituted in 1 mL of 20% ACN in water. Samples were analyzed using a Shimadzu Nexera LC-30AD with MS-8050 with a SelectraCore® **DA** UHPLC Column (100 mm x 2.1 mm, 2.7 μ m). Mobile phase A was 0.1% formic acid in water and mobile phase B was ACN. Free steroid compounds were analyzed in positive mode while the sulfate steroid conjugates were analyzed in negative mode.

Results: Analytes were successfully extracted from urine and blood with high recoveries and low matrix effects at a concentration of 25 ng/mL (n=5). Extraction recoveries of analytes from urine ranged from 89-95% and the matrix effect for all analytes was within \pm 25%. Bias and precision for urine samples were both \leq 5%. Extraction recoveries of analytes from blood ranged between 71-102% and the matrix effect for all analytes was within \pm 25%. Bias and precision for urine samples were both \leq 20%. The method has a LOD of 0.5 ng/mL and a LOQ of 1 ng/mL.

Conclusion/Discussion: Both free and sulfated steroids were successfully extracted from urine and blood by utilizing the Styre Screen[®] HLB SPE cartridges with excellent recoveries, bias, and precision. These results indicate that this optimized method for analyzing free and intact steroid sulfates is highly efficient and can be readily implemented in high-throughput laboratories.

P60 - Testing for drugs of abuse in human blood to ANSI/ASB120 standards by high-resolution, accurate-mass mass spectrometry

Kristine Van Natta, Courtney Patterson, and **Stephanie Samra***; Thermo Fisher Scientific, San Jose, CA.

Background/Introduction: As specified by ANSI/ASB120, testing for drugs of abuse testing in blood is an essential and routine requirement when investigating impaired driving, drug-facilitated crimes, or deaths. Blood samples collected by investigators are typically sent to a toxicology, crime, or coroner's laboratory for testing. The analyst must quickly and accurately detect and identify compounds of interest with unflinching confidence. The number of available drugs and toxins are growing at an alarming rate and vary widely in chemical hydrophobicity and chemical structure, making these analyses a true analytical challenge.

Liquid chromatography coupled with mass spectrometry (LC-MS) offers a solution for detecting these compounds with a combination of specificity and sensitivity not achievable by standard immunoassay methods. However, in order to screen for compounds by LC-MS, the separation must ensure retention and separation of chemically diverse compounds and the subsequent MS analysis must not only detect and identify but also confirm identified compounds with confidence. Here we present a method for simultaneous screening for ten opioids in blood by liquid chromatography and mass spectrometry.

Objectives: Simultaneously detect, identify, and confirm ten opioid drugs from the ANSI/ASB120 panel in blood by liquid chromatography and high-resolution, accurate-mass (HRAM), data-dependent MS² mass spectrometry.

Methods: Ten non-labeled opioid standards—6-acetylmorphine, buprenorphine, codeine, fentanyl, hydrocodone, methadone, morphine, norbuprenorphine, oxycodone, and tramadol—were spiked into 100 μ L of whole blood and precipitated with 150 μ L of 0.1 M zinc sulfate and 250 μ L of methanol. Samples were centrifuge and the supernatant was diluted with water. Aliquots spanning a concentration range from 400 ng/mL down to 0.05 ng/mL were injected in triplicate to determine screening and confirmation cutoffs.

Analytes were separated using a 15-minute gradient elution on a Thermo Scientific[™] Vanquish[™] Flex ultra-high performance liquid chromatography (UHPLC) system. Mobile phases A and B consisted of 2 mM ammonium formate with 0.1% formic acid in water and in acetonitrile:methanol (1:1, v:v), respectively. The separation was carried out on a Thermo Scientific[™] Accucore Phenyl-Hexyl column (2.6 µm, 100 x 2.1 mm).

Targeted screening and quantitation were performed on a Thermo Scientific^M Orbitrap Exploris^M 120 mass spectrometer. Full-scan, targeted, data-dependent MS/MS scanning was used with an inclusion list for the targeted compounds. The inclusion list contained the exact masses of the opioids, their ionization polarities, and retention times. Resolutions of 60,000 (FWHM at m/z 200) for full scan and 15,000 for MS² were employed. An isolation window of m/z 1.5 and stepped collision energies were applied to generate rich HRAM MS² spectra.

Results: Limits of detection (LOD), quantification (LOQ), and identification (LOI) were determined for the opioids by processing the data with Thermo Scientific[™] Trace Finder[™] 5.1 software. LOQ was defined as the back-calculated concentration where % difference was less than 30, LOD was defined as a peak present with a mass accuracy of < 5ppm, and LOI was defined as a passing isotopic pattern score of 70 or above, expected fragment ions present, and an MS² library match score of 70 or above. LOQs for the opioids were 0.25 ng/mL except for buprenorphine (0.5 ng/mL), methadone (0.1 ng/mL), norbuprenorphine (0.1 ng/mL), and tramadol (0.1 ng/mL). These were the same as LODs for all opioids except buprenorphine (0.25 ng/mL). All opioids passed ANSI/ASB120 screening and confirmation requirements except norbuprenorphine which passed for isotope pattern and fragment ions but did not have a passing library match score.

Conclusion/Discussion: A comprehensive LC-HRAM-MS/MS workstream was used to screen for ten opioids in whole blood. All screening and confirmation requirements of ANSI/ASB120 were met with the single exception of norbuprenorphine, which failed the library search score needed for LOI.

P61 - Distribution of para-fluorofentanyl across multiple matrices in two postmortem cases

Denice Teem*¹, Daniel Isenschmid², Kanubhai Virani¹, Carl Schmidt³, Barry Logan². ¹ Oakland County Medical Examiner's Office, Pontiac, MI. ² NMS Labs, Horsham, PA. ³ Wayne County Medical Examiner's Office, Detroit, MI.

Background/Introduction: Para-Fluorofentanyl (4-Fluorofentanyl, p-FF) is a fluorinated fentanyl derivative in the para position of the aniline ring. It is an opioid analgesic that is currently listed as DEA Schedule I in the United States and is reported to be approximately 3 times less potent than fentanyl. Although synthesized in the 1960's, p-FF had brief popularity in the 1980's and has once again re-emerged in positivity in forensic casework since late 2020, often in combination with other drugs. p-FF prevalence is believed to be increasing as a result of limited availability of traditional precursor chemicals used to make fentanyl. Currently, limited information is available demonstrating the distribution of p-FF in biological samples of forensic interest where polypharmacy is not present.

Objectives: To quantitate the postmortem p-FF concentrations in all available matrices collected at autopsy for two individual cases from Southeast Michigan where p-FF was listed as contributing to cause of death.

Methods: Analysis for p-FF was performed by Liquid Chromatography Tandem Mass Spectrometry (LC-MS/MS) with a reporting limit of 0.050 ng/mL. The method was validated in compliance with the ASB Standard for method validation.

Results: These two deaths occurred in neighboring counties of metro Detroit 10 days apart. It is unknown if there is any correlation between the decedents or the source of the drug material.

Case 1	Source femoral blood (gray top tube)	para-Fluorofentanyl concentration (ng/mL) 7.9		
	femoral blood (gray top tube)	6.6		
	femoral blood (lavender top tube)	6.1		
	femoral blood (red top tube)	5.9		
	vitreous fluid	2.0		
	urine	8.0		
	gastric contents	220		
Case 2	peripheral blood (gray top tube)	14		
	peripheral blood (gray top tube)	13		
	vitreous fluid	6.0		
	urine	118		
	liver tissue	210 ng/g		

Conclusion/Discussion: Most commonly, synthetic opioids and novel psychoactive substances (NPS) are found in forensic casework in combination with other drugs of abuse such as cocaine, fentanyl, and heroin. However, it is important that medical examiners and coroners are aware that NPS cannot always be detected by routine drug screens. It is recommended that laboratories have the capability to monitor for NPS when more common drugs of abuse are not present or at concentrations not believed to be contributory to cause of death.

Initial testing of both cases in this study included an Expanded Postmortem Forensic Toxicology panel on femoral blood in Case 1, and peripheral blood in Case 2, as well as a urine screen in Case 2. Due to the lack of anatomical findings at the autopsy and no common drugs of abuse found on the initial testing to explain the cause of death, additional testing for designer opioids was performed in both cases to confirm the presence of p-FF in blood. Approximately six months later, subsequent testing for fentanyl, norfentanyl, 4-ANPP and designer opioids was performed on the remaining matrices to demonstrate the distribution of p-FF in a variety of postmortem specimens and preservative types. p-FF was detected in all matrices analyzed which suggests that alternative specimens are a viable option to test for p-FF when blood is not available at autopsy or when samples are stored at refrigerated temperatures but cannot be analyzed immediately after autopsy.

P62 - Performance of LC-Ion Trap-MS screening in forensic toxicology – a seven-year recap using proficiency test data of the Toxtyper®

Jürgen Kempf*, Christa Pelz, Tobias Leupolz, Laura M. Huppertz

Medical Center - University of Freiburg, Institute of Forensic Medicine, Freiburg, BW, Germany.

Background/Introduction: Liquid chromatography - mass spectrometry (LC-MS) is one of the most frequently used analytical techniques in forensic toxicology. Comprehensive screening methods often include several hundred analytes, theoretically allowing the detection and identification of compounds of different drug classes and a wide range of physico-chemical properties. Besides the technical possibilities of the instrument, the complete workflow – especially sample preparation – effects the detection of analytes in matrix. Besides the respective identification criteria, the operator must know the possibilities and - more important - limitations of the workflow. One piece of quality assurance is the use of certified proficiency tests.

Objectives: This work aims at evaluating the performance of the LC- ion trap -MS screening workflow in our lab and identifying unknown blind spots and possible improvements.

Methods: Two commercial proficiency tests (Arvecon GmbH, Walldorf, Germany), organized on in cooperation with the German Society of Toxicological and Forensic Chemistry (GTFCh) were used for this evaluation. One test requires the identification of compounds in urine (UF), the other (QSA) includes a made-up case scenario with corresponding blood and urine specimens.

The screening is an LC-ion trap-MS approach (Toxtyper[®], Bruker Daltonik, Bremen, Germany) with an in-house extended spectral library including around 1000 compounds. Serum samples (1 mL) are extracted using alkaline liquid-liquid extraction, urine samples (100 µL) were precipitated with cold acetonitrile prior to analysis.

Results: There were three annual UF and QSA proficiency tests (2015 – 2021), resulting in data of 21 serum and 42 urine samples.

<u>UF:</u> 21 urine samples had been spiked with 58 different compounds to a total of 156 required findings. 86.5 % of the spiked compounds could be identified correctly. 21 missed identifications can be classified as followed: Three analytes (GHB, MDPV, felbamat) were not included in the method at the time of the analysis (7x). Two analytes (EtG, norbuprenorphine) are included in the library but known not to be detectable with that workflow (6x). Six analytes (methamphetamine, tramadol, imipramine, acetylmorphine, pregabaline, methoxethamine) were true false negatives (8x), resulting in a false negative rate of 5.1 %.

<u>QSA</u>: 42 samples had been spiked with 50 different compounds to a total of 137 possible findings and there was only a low correct positive rate of 63.5 %. Missed compounds could be categorized as follows: 15 compounds not detectable by LC-MS screening due to chemical properties (e.g. cyanide, formic acid, ethanol) or not included in the method (e.g. Dehydronorketamine or chlormethiazole metabolite) (28x), two compounds (EtG and EtS) not suitable for reliable detection with this workflow (6x), seven compounds (e.g. pregabalin or THC-COOH) only detectable in one of the two matrices (12x), and three compounds (THC, pregabalin and MDA) were false negatives (4x).

Seven cases would have been assessed incorrectly if this approach would have been the only analytical method applied. Taking into account the limitations described above, there remains one case with false negative findings (pregabalin) in both matrices that would have led to a misinterpretation of this case.

Conclusion/Discussion: The evaluated method is one important tool for the systematic toxicological analysis in DUID and both clinical and postmortem intoxication cases, especially when prescription drugs are involved. Data of these proficiency tests lead to constant addition of analytes and improvement of detection criteria. Although a wide range of analytes can be covered, there are known limitations, e.g. due to psysico-chemical properties of compounds, requiring additional approaches like immunoassays, GC-MS and target LC-MS analyses. Besides analyzing fortified matrices or comparing screening results with the subsequent quantitative results, the analysis of certified proficiency tests is an effective and crucial way to assure the quality of a screening workflow.

P63 - Separation and detection of isomeric cannabinoids and metabolites by LC-MS/MS

Kaitlyn B. Palmquist, PhD*; Crystal Arndt, MSFS, D-ABFT-FT; Teresa R. Gray, PhD, F-ABFT. Harris County Institute of Forensic Sciences, Houston, TX, USA.

Background/Introduction: While cannabis legalization status varies from state to state, a general progression toward full legalization of recreational and medicinal use is apparent. With variation in legality and ambiguity in the 2018 Farm Bill, the cannabis industry has grown to incorporate, manufacture, and market isomers of Δ^9 -tetrahydrocannabinol (Δ^9 -THC), the main psychoactive component of marijuana. While these isomers have the same molecular formula, the legality and psychoactivity associated with individual chemical structures varies, thus highlighting the importance of differentiation for identification and interpretation performed by forensic toxicologists.

Objectives: The goal of this project was to improve cannabinoid detection through efficient chromatographic separation of isomeric compounds using liquid chromatography-tandem mass spectrometry (LC-MS/MS) for practical applications.

Methods: Target analytes were Δ^9 -tetrahydrocannabinol (Δ^9 -THC), Δ^8 -tetrahydrocannabinol (Δ^8 -THC), $\Delta^{9,11}$ -tetrahydrocannabinol (Exo-THC), cannabidiol (CBD), 11-hydroxy- Δ^9 -THC (11-OH-THC), 11-nor-9-carboxy- Δ^9 -THC (THC-COOH), 7-hydroxy-cannabidiol (7-OH-CBD) and 7-carboxy cannabidiol (7-COOH-CBD). Blood (0.25 mL), liver homogenate (0.25 mL of 4x dilution) and urine samples (0.25mL) were extracted using supported liquid extraction. Separation of isomers was achieved using a Phenomenex[®] Kinetex[®] PFP column (100 mm x 3 mm x 2.6 µm). with similar phase guard and a gradient mobile phase system consisting of 0.1% formic acid in water (A) and 0.1% formic acid in methanol (B) at a flow rate of 0.6 mL/min. Samples were analyzed using an Agilent Technologies 1290 Infinity II liquid chromatograph coupled to an Agilent Technologies 6470 triple quadrupole mass spectrometer. Data were acquired using positive electrospray ionization and multiple reaction monitoring (MRM).

Results: The presented method successfully and efficiently separated all target analytes with an 11 min total run time. Isomeric forms of THC (Δ^9 -THC, Δ^8 -THC, exo-THC, and CBD) were baseline resolved, mitigating the potential for interferences.

Conclusion/Discussion: Commercialization of cannabis products containing THC isomers continues to expand in the United States. With the emergence of new legal compounds, such as Δ^{10} -THC, the presented method provides promise for expansion as commercially available reference materials become available. To better understand and interpret the impact on human performance, forensic toxicologists need to be able to differentiate isomeric cannabinoids in biological specimens. The presented method successfully separated and detected four THC isomers (including exo-THC, a potential interferent) and metabolites using LC-MS/MS and has practical applicability to driving under the influence of drug, drug-facilitated crime, and medico-legal death investigations.

P64 - The use of isotopically labeled standards in a multipoint internal calibration (MPIC) for the quantification of amphetamine in biological specimens by LC-MS

Katherine Gussenhoven^{1*}, Mackenzie Smith¹, Florine Sanchez¹, Gabrielle Gazette¹, Alyssa Queen¹, Szabolcs Sofavi, MSChE², Jocelyn V. Abonamah, MFS³ and Harold E. Schueler, Ph.D.¹. ¹Ohio Northern University, Ada, OH; ²Cuyahoga County Medical Examiner's Office, Cleveland, OH; ³Cleveland Clinic Foundation, Cleveland, OH.

Background/Introduction: Isotopologues are almost chemically identical drugs whose only identifiable difference in the atomic mass through labeling with heavier, but still naturally occurring isotopes/atoms such as deuterium (2H or D) or carbon 13 (13C). Currently, isotopically labeled drugs are used as internal standards in toxicological quantification methods, but there is potential to use them as internal calibrators. In a traditional procedure, the area ratio of amphetamine to its internal standard is found for multiple known concentrations of amphetamine, a calibration curve is constructed and then used to quantify amphetamine in unknown specimens. These isotopically labeled compounds have negligible effects on the unlabeled drug concentration and are distinguishable by mass spectrometry from their unlabeled form. Using an LC-MS quantification of amphetamine is made possible through the comparison of the areas associated with

amphetamine's [M+H]+ peak, 136 atomic mass units, in comparison to that of its isotopologues whose [M+H]+ peaks are correspondingly greater. Multiple deuterated standards would be able to be differentiated from each other in accordance with their [M+H]+ peaks. Thus, spiking different isotopically labeled standards in the same sample at different concentrations will provide internal calibrators in a method called Multipoint Internal Calibration (MPIC).

Objectives: To propose a new MPIC technique using isotopically labeled standards (e.g., D) as a means of quantifying amphetamine in a single forensic sample using LC-MS analysis.

Methods: Using an LC-MS, deuterated amphetamine standards were run individually to identify the retention times and interference of each standard; as well as a single calibrator solution consisting of four different concentrations (i.e., D5 at 10 ng/mL, D6 at 100 ng/mL, D8 at 500 ng/mL, and D11 at 1000 ng/mL) of deuterated amphetamine standards that was prepared to generate calibration curve via MPIC. The Cuyahoga County Medical Examiner's Office's validated traditional procedure for the quantification of amphetamines was used to investigate the new method. A sheep's blood liquid-liquid extraction was performed using the traditional external calibration method of quantification on spiked samples at known concentrations of amphetamine extracts. These results were compared to an identical liquid-liquid extraction performed using the MPIC to create comparable calibration curves and assess the accuracy of each method.

Results: The isotopically labeled standards contribute to each other's [M+H]+ peaks. The MPIC procedure generates a calibration curve that has comparable characteristics to calibration curves constructed using external calibrators. When compared to the accuracy of the amphetamine concentration predicted by the calibration curves generated by the traditional external calibration method, the MPIC procedure has shown less accuracy, hypothesized to be the result of deuter-ated standard impurities.

Conclusion/Discussion: Amphetamine can be quantified in a single sample using a new technique of MPIC using isotopically labeled (i.e., deuterated) standards. This method has the potential to replace traditional external calibration techniques and drastically improve sample turn-aroundtimes (TATs). This procedure eliminates the confounding matrix effects and compensates for ion suppression/enhancement associated with LC-MS methods due to the isotopologues being chemically identical and in the same sample. Current difficulties in quantification are due to the presence of impurities in isotopically labeled standards as well as an acetonitrile cluster interfering with the quantification of amphetamine-D5. The extraction process may increase impurities in acidic extraction conditions due to the possibility of proton exchange, as such carbon 13 labeled standards should be used in future studies. Additionally, using ethanol or methanol as part of mobile phase B is a proposed solution to the cluster quantification issue. Further studies would address these issues.

P65 - Separation and quantitation of natural and unnatural THC isomers and analogues by high-performance liquid chromatography tandem mass spectrometry (HPLC-MS/MS)

Kimberly N. Karin^{1*}, Alaina K. Holt², Emani R. Smith², Sean Orlowicz³, Adrian M. Taylor⁴, Linda Prengaman⁵, Carl E. Wolf^{2,6}, Grace R. Williams⁶, Michelle R. Peace², Justin L. Poklis¹.¹Department of Pharmacology and Toxicology, ²Department of Forensic Science, Virginia Commonwealth University, Richmond, VA, ³Phenomenex, Torrance, CA, ⁴Sciex, Ontario, Canada, ⁵Sciex, Framingham, MA⁶Department of Pathology, Virginia Commonwealth University, Richmond, VA.

Background/Introduction: As a result of the overproduction of cannabidiol (CBD), retailers converted CBD to Δ^9 -tetrahydrocannabinol (THC) isomers and analogues. Commercially available products advertise the presence of THC isomers including $\Delta^8 \Delta^{6a,10a} \Delta^{10}$ -THC, and other analogues such as hexahydrocannabinol (HHC), tetrahydrocannabiphorol (THCP), and THC-O (acetate). Some of these compounds may be found naturally in plant material, but at negligible concentrations compared to the reported doses of marketed products. While the potency, efficacy, and adverse effects of these emerging compounds are relatively unknown, early reports indicate these analogues may produce hallucinations, sedation, vomiting, and seizures. To monitor these emerging cannabinoid analogues, a method for their chromatographic separation and quantitation is necessary.

Objectives: To develop a HPLC-MS/MS method for the separation and quantitation of emerging THC isomers and analogues and to analyze commercially available e-liquids.

Methods: A method for the separation of THC isomers and analogues was developed using a Sciex ExionLC 2.0 liquid chromatograph attached to a Sciex 6500 QTRAP system with an IonDrive Turbo V source for TurbolonSpray. Reference material for the acetate analogues of the cannabinoids were synthesized from certified reference material by heating in pyridine and acetic anhydride at 70-75°C overnight. Conversion of the parent to the acetate form was confirmed by gas chromatography mass spectrometry. Calibration curves from 2.5 to 250 mg/mL for the cannabinoids were prepared, along with low, mid, and high controls at 7.5, 75, 187.5 mg/mL. A total of 26 e-liquids labeled to contain THC isomers and analogues were purchased. The e-liquids (40 mg) were diluted and agitated using a Beadruptor 24 (Biotage, Uppsala, Sweden). Chromatographic separation was performed on a Phenomenex Kinetex* **2.6µm C18 100Å 150 x 3mm** column with an isocratic method of 75% acetonitrile and 25% water containing 1 g/L ammonium formate and 1% formic acid with a flow rate of 0.650 mL/min. The following transitions were monitored in multiple reaction monitoring for the analytes and deuterated internal standards: cannabichromene/ Δ^9 -THC/ Δ^8 -THC- $OL\Delta^{9}$ -THC-O 357>123 & 315>193; CBD-di-O 399>193 & 399>314; CBD-d₃/THC-d₃ 318>123 & 318>193; Δ^9 -THC-O-d₃ and CBD-di-O-d₃ 402>193 & 402>317.

Results: The method separated the THC Isomers and/or isobars. The linearity of all the analytes had r² values \geq 0.9958. The bias was \leq 19 % with coefficients of variation (%CV) \leq 15% of for all controls. No carryover was observed in the analyte free controls. All 26 e-liquids contained one to six of the analytes of interest. Of the samples, 18 contained Δ^8 -THC in combination with HHC, THC-Ps and/or THC-Os. HCC (12-490 mg/mL), THC-Ps (4-13 mg/mL) and THC-Os (13-540 mg/mL), $\Delta^{6a,10a}$ -THC (6-200 mg/mL) were found in 8, 8, 14 and 5 e-liquids, respectively.

Conclusion/Discussion: A HPLC-MS/MS method for the separation and quantitation of emerging THC isomers and analogues including HHC, Δ^{8} -THC, $\Delta^{6a,10a}$ -THC, THC-P, and THC-O was developed. Little is known about the pharmacology and toxicology of these emerging THC isomers and analogues, posing a risk to public health and safety. It is imperative for laboratories to be able to separate and analyze these THC isomers and analogues. Without a method for the separation of the isomers, these compounds may be falsely reported as Δ^{9} -THC. This HPLC-MS/MS method allows for the monitoring of the prevalence of these compounds in products and toxicological cases.

Funding: Funded in part by National Institute of Health (P30DA033934 and T32DA007027) and National Institute of Justice 2019-MU-MU-0007.

P66 - Detection of an expanded SAMSHA-7 pre-employment panel by LC-MS/MS

Kirstin Faoro*, Kristine Van Natta, Richard Gibson Thermo Fisher Scientific, Inc., San Jose, CA.

Background/Introduction: A highly sensitive and specific LC-MS/MS analytical method has been developed for the quantitation of 19 common Drugs of Abuse (DoA) that included-- 6-Acetylmorphine (6-AM), Amphetamine, Cocaine metabolite (Benzoylecgonine) (BZN), Codeine, Hydrocodone, Hydromorphone, Methylenedioxyamphetamine (MDA), N-Methyldiethanolamine (MDEA), Methylenedioxymethamphetamine (MDMA), Methamphetamine, Morphine, Naloxone, Norcodeine, Norhydrocodone, Oxycodone, Oxymorphone, Phencyclidine (PCP), Phentermine, and Marijuana metabolite (THCA) in urine. A 4-minute reverse phase liquid chromatography tandem mass spectrometry method was used to achieve the required sensitivity and dynamic ranges outlined in the Substance Abuse and Mental Health Services Administration (SAM-SHA) mandatory guidelines for federal workplace drug testing programs with simple dilute and shoot sample preparation.

Objectives: Demonstrate the sensitivity and selectivity of the Thermo Scientific[™] TSQ Quantis Plus[™] mass spectrometer for fast accurate quantitation of the expanded SAMSHA-7 pre-employment panel.

Methods: A liquid-chromatography-mass spectrometry method was developed using Cerilliant[™] certified reference materials (Round Rock, TX) for the quantitation of the expanded SAMSHA pre-employment panel. Standards were resuspended in methanol, spiked into blank urine matrix, and diluted 20x for 14 different concentration levels representing a range of 0.1 ng/mL to 2000 ng/mL in sample.

The chromatographic method used for separation was a 4 minute reverse phase gradient run at 0.55 mL a minute on a Thermo Scientific[™] Vanquish Flex[™] system consisting of a binary pump, a column oven, and a temperature controlled autosampler. The separation was performed on a Thermo Scientific[™] Accucore Biphenyl 50 x 2.1mm, 2.8µm column maintained at 35 °C. Mobile phases consisted of 0.1% formic acid with 5mM ammonium formate in water for mobile phase A and 0.1% formic acid with 5mM ammonium formate in methanol for mobile phase B. The limit of detection (LOD) was defined as the lowest concentration for which a peak was observed with a S/N of at least 3, within 0.2 min of the expected retention time (RT), had a %CV of at least 15 within each batch across 5 injections. The limit of quantitation (LOQ) is defined as the lowest concentration for which quantitation bias was <20% while also meeting the detection criteria of the LOD.

Results: The 4 min method allowed for separation for common isobars such as Morphine /Hydromorphone and Methamphetamine /Phentermine. Good linearity and reproducibility were obtained across the dynamic range of the 19 DoAs and with a coefficient of determination R2>0.99 for all compounds. The lower limits of detection (LLOD) and lower limit of quantitation (LLOQ) were determined to range from 0.1 to 10 ng/ml and excellent reproducibility was observed for all compounds (CV < 15%). A partial validation in accordance of SAMSHA guidelines was conducted proving accuracy and precision of the method while establishing an upper limit of linearity (ULOL) and carryover thresholds while testing for matrix interferences.

Conclusion/Discussion: A sensitive, simple, specific, and accurate liquid chromatography mass spectrometry method was developed on the and verified for the simultaneous measurement of an expanded SAMSHA-7 panel to include various isobars that are commonly seen as interferences.

P67 - Improved THCCOOH isomer separation through alternate column selectivity

Larissa Karas^{1*}, Erin L. Karschner². ¹Department of the Army, Fort Meade, MD 20755; ²Armed Forces Medical examiner System, Division of Forensic Toxicology, Dover AFB, DE 19902.

Background/Introduction: 11-nor-9-carboxy-delta-9-tetrahydrocannabinol (Δ 9-THCCOOH) is the most frequently detected illicit drug metabolite in the military random urine drug testing program. Over the past year, military drug testing laboratories began testing and reporting Δ 8-THCCOOH in addition to the Δ 9 isomer. The Fort Meade Army Drug Testing Laboratory's original method provided adequate resolution between peaks in over 93% of reported cases. However, approximately 6% of samples contained more extreme ratios of isomers (e.g., 2,000 ng/mL Δ 8-THCCOOH to 15 ng/mL Δ 9-THCCOOH), resulting in poor resolution for the less abundant peak. These issues with poor resolution result in one isomer able to be reported, while the other is reported as invalid. The Department of Defense standard for reporting drug testing results requires accurate information regarding the THCCOOH isomers present in specimens; hence, laboratories are encouraged to develop methods with greater separation, while maintaining analytical runtimes required for a high-throughput laboratory.

Objectives: Develop and validate a quantitative method with optimal separation of $\Delta 8$ - and $\Delta 9$ -THCCOOH.

Methods: Automated sample preparation and extraction was performed on a Hamilton MicroLab Star. 500 μ L urine was hydrolyzed and extracted using strong anion exchange tips from DPX Technologies. Tips were conditioned with 50% methanol (MeOH) and washed twice after sample loading (once with 84:15:1 water:acetonitrile(ACN):ammonium hydroxide and once with 50:50 MeOH:ethyl acetate). Analytes were eluted using 4% formic acid in ACN. Eluents were dried down and reconstituted in 75 μ L mobile phase and extracts analyzed by LC-MS/MS using an Agilent 1290 Infinity LC coupled with a 6460 triple quadrupole mass spectrometer operating in negative mode. Isocratic separation was conducted using a Restek Raptor FluoroPhenyl 2.7 μ m, 100 x 2.1 mm column for a total runtime of 8.5 min. Quantitation was performed using Δ 9-THCCOOH-D9, with two multiple reaction monitoring (MRM) transitions collected for each analyte and internal standard.

Results: The quantitative urine method was validated for linearity, precision and accuracy, specificity (exogenous interferences), matrix effects (ion suppression/enhancement), carryover, and dilution integrity. Linearity was demonstrated from 3 to 1,000 ng/mL with R² values greater than 0.998. Intra- and inter-day precision and accuracy were acceptable at less than 10% CV and less than 15% bias for both analytes. No exogenous interferences were observed, and the internal standard normalized ion suppression/enhancement was less than 10% for each analyte. No significant carryover was observed when tested up to 30,000 ng/mL. Dilution integrity was acceptable up to 50x.

Separation efficiency of the $\Delta 8$ - and $\Delta 9$ -THCCOOH isomers was vastly improved from the laboratory's original method, which utilized an Agilent Poroshell 120 C18 stationary phase column. The total runtime of the method was 8.5 min and achieved the desired resolution of one min between peaks. However, runtime could be reduced to 3.5 min if only baseline separation is desired.

Conclusion/Discussion: The fluorophenyl stationary phase provided an alternate selectivity to traditional C18, which has been used by previously published methods to separate THC and THCCOOH isomers. This method modification highlights the utility of investigating different column stationary phases during method development to improve separation and decrease analysis time. Increased resolution of THCCOOH isomers is able to be achieved for laboratories with strict reporting criteria. This validated method, when implemented in a high-throughput urine drug testing laboratory, reduced the percentage of samples where one peak was not adequately resolved from 6% to zero.

P68 - Fast Derivatized LC-MS/MS Method for separation and quantitation of amphetamine and methamphetamine

Linda Smith¹*, Sarah Aijaz¹, Uma Sreenivasan¹, ¹Millipore Sigma, Round Rock, TX.

Background/Introduction: Amphetamines are frequently used to treat ADHD and narcolepsy due to its psychostimulant properties. Both amphetamine and methamphetamine contain chiral centers and exist as enantiomers dextro- and levo. Racemic mixtures are used in prescription drugs whereas dextro- has been found to be the main constituent in illegal drugs due to its stronger potency. The ability to distinguish between these enantiomers is crucial to forensic laboratories in identifying illicit use.

Objectives: We present a 4-minute LC-MS/MS method that accomplishes enantiomeric separation and accurate quantitation of both amphetamine and methamphetamine without the use of a chiral column by utilizing a fast and simple derivatization.

Methods: Samples consisted of d- and l-amphetamine and d- and l-methamphetamine spiked urine. Deuterium labeled amphetamine and methamphetamine were used as internal standards. A calibration curve was prepared from certified reference standards (Cerilliant Corporation). Derivatization with 0.1% (w/v) Marfey's reagent was achieved by incubation for one hour at 45 °C, quenched with 1M HCl, dried and reconstituted in water:methanol (40:60). A Supelco Ascentis Express F5 100 x 3.0, 2.7µm column was used to separate each of the enantiomers of amphetamine and methamphetamine. Samples were analyzed in negative mode and targeted MRM using the Agilent 6495 QQQ mass spectrometer, equipped with an Agilent 1290 UHPLC system. Mobile phases constituted 0.1% formic acid in water and 0.1% formic acid in methanol. Method validation studies include accuracy, precision, LOD/LLOQ, carry over and matrix effects. Processed sample stability and upper limit of linearity were also evaluated in this study.

Results: This method can be used to quantitate each enantiomer of amphetamine and methamphetamine under four minutes with a LLOQ of 50 ng/mL. Enantiomeric peaks of amphetamines and methamphetamines were resolved with co-elution of the internal standard. No carryover or interferences were detected. Linear regression coefficients (r^2) for each analyte were greater than 0.99.

Conclusion/Discussion: A simple, high throughput method for the quantitation of d- and l-amphetamine and methamphetamine by LC-MS/MS was developed and proved rugged. This method allows for selective and specific detection without the use of chiral columns over a linear range of 50-5000 ng/mL.

P69 - Generation and trapping of reactive drug metabolites by a thiol-containing hemoglobin peptide

Ludmyla S. Tavares, M.S.*, Florida International University, Miami, FL, Anthony P. DeCaprio, Ph.D., Florida International University, International Forensic Science Research Institute, Miami, FL. *

Background/Introduction: Currently, long term assessment of drug abuse or exposure is limited to hair analysis, which presents numerous challenges. An alternative approach is the study of covalent binding of electrophilic drug metabolites to proteins and/or peptides, such as hemoglobin (Hb) and glutathione, to yield drug-protein or drug-peptide "adducts". Such adducts generally survive throughout the life of the protein (~120 days in the case of human Hb), and their measurement can therefore reflect retrospective or cumulative drug exposure during this extended time period.

Objectives: In this study, as a preliminary to whole Hb protein work, the capability of electrophilic drug metabolites to form adducts with a specific b-Hb tryptic peptide containing a nucleophilic free thiol moiety (*i.e.*, b^{93} Cys peptide; GTFAT-LSELH⁹³CDK) was investigated. Drugs selected for analysis included acetaminophen (APAP), cocaine (COC), 3,4-methylene-dioxymethamphetamine (MDMA), methamphetamine (METH), and Δ^9 -tetrahydrocannabinol (THC). Peptide analysis was performed using HRMS on an Agilent 1290/6530 LC-QTOF-MS system.

Methods: For the generation of reactive metabolites and binding to peptide, two methods were evaluated: an *in vitro* metabolic trapping assay using human liver microsomes (HLM) and an electrochemical (EC) oxidation assay. The HLM assay used was adapted from a previously developed method in the PI lab. Briefly, all assay components (HLM, NADPH, G6P, G6PD, b⁹³Cys peptide, and drug) were combined in ammonium bicarbonate buffer in a microfuge vial and vortexed, followed by an incubation of 4 h at 37°C. Vials were then centrifuged and aliquots of supernatant transferred to the LC for analysis. The EC oxidation assay was performed using a system of three electrodes: Pt mesh as the working electrode, calomel standard as the reference electrode, and platinum rod as the counter electrode. Predicted covalent thiol modifications for each drug and putative metabolites were added as target ions to BioConfirm B.08.00 software via Sequence Manager and were used to determine the peptide modifications. For the Targeted MS/MS studies, the spectra were collected, compared to the theoretical peak list supplied by Protein Prospector software, and confirmed peaks were recorded.

Results: The results for the HLM assay showed the formation of three covalent thiol adducts with APAP, one with COC, two with MDMA and one with METH. For the electrochemical oxidation, three adducts were formed with APAP, one with COC, one with MDMA, one with METH and one with THC. Drug-b⁹³Cys peptide adducts were found with both methods for the following drugs and metabolites: APAP and metabolites (N-acetyl-p-benzoquinone imine and 1,4-benzoquinone), COC and metabolite (ecgonidine), MDMA and metabolites (3,4-dihydroxy amphetamine, and an aminochrome derivative), METH metabolites (methcathinone and benzoic acid), and THC and metabolites (11-COOH-THC and carbaldehyde-THC). Results were confirmed by MS and MS/MS analyses.

Conclusion/Discussion: Results of the *in vitro* trapping assay studies with the b^{93} Cys peptide revealed that both HLM and EC oxidation approaches can be employed in the generation and trapping of metabolites by peptides containing reactive thiol moieties. The ability of these drugs and their metabolites to bind specifically to the β -Hb peptide containing 93 Cys suggests that these moieties could be monitored in intact Hb as longer-term exposure biomarkers for selected drugs. Additional work is testing the applicability of this approach for detecting such specific Hb modifications in authentic specimens.

P70 - The use of isotopically-labeled standards in a multi-point internal calibration (MPIC) for the quantification of methamphetamine in biological specimens by LC-MS

Mackenzie Smith^{1*}, Katherine Gussenhoven¹, Florine Sanchez¹, Alyssa Queen¹, Gabrielle Gazette¹, Szabolcs Sofalvi, MSChE², Jocelyn V. Abonamah, MFS³ and Harold E. Schueler, Ph.D.¹.. ¹Ohio Northern University, Ada, OH; ²Cuyahoga County Medical Examiner's Office, Cleveland, OH; ³Cleveland Clinic Foundation, Cleveland, OH. *

Background/Introduction: According to the National Institute of Health methamphetamine is one of the most commonly abused drugs. This highly addictive and potent stimulant is a great candidate for the development of a new quantitative extraction technique. This new method achieves shorter batch run times due to the elimination of external calibrators, as well as the need to evaluate processed sample stability, and this method does not suffer from the matrix effects or ion suppression/enhancement associated with LC-MS. Methamphetamine has a molecular ion $[M+H]^+$ of 150 *m/z*. Spiking multiple different isotopologues of methamphetamine; such as: $D_5 [M+H]^+$ of 155 *m/z*, $D_8 [M+H]^+$ of 158 *m/z*, $D_9 [M+H]^+$ of 159 *m/z*, $D_{11} [M+H]^+$ of 161 *m/z*, and $D_{14} [M+H]^+$ of 164 *m/z* in a single case sample at increasing concentrations will produce an internal calibration curve which can be used to quantify the amount of methamphetamine within the same sample.

Objectives: Introduce the use of Multi-Point Internal Calibration (MPIC) to quantify drugs in forensic specimens, which reduces the turn-around-time (TAT) for each case, eliminates matrix-effects when using decomposed specimens, and decreases validation parameters associated with the use of an external calibration, such as ion suppression/enhancement and processed sample stability.

Methods: To quantify methamphetamine using both MPIC and traditional external calibration methods, 0.5 mL of sheep's blood was used for each sample. A working solution of the MPIC calibration mixture containing five different methamphetamine isotopologues at five concentration levels ($D_5 = 10 \text{ ng/mL}$, $D_8 = 100 \text{ ng/mL}$, $D_9 = 300 \text{ ng/mL}$, $D_{11} = 600 \text{ ng/mL}$, and $D_{14} = 1,000 \text{ ng/mL}$), was used to generate the calibration curves. Using an established protocol validated by the Cuyahoga County Medical Examiner's Office, 50 µL of the MPIC solution was spiked into each sample, and a liquid-liquid extraction was performed. To evaluate the results of the MPIC trials, concurrent extractions using the traditional external calibrator procedure were conducted. The traditional extractions used the same liquid-liquid extraction protocol as the MPIC extraction with the following differences: methamphetamine- D_5 was used as the internal standard, and the external methamphetamine calibrator concentrations were: 10 ng/mL, 100 ng/mL, 300 ng/mL, 600 ng/mL, and 1,000 ng/mL. A ThermoScientific Dionex UltiMate 3000 HPLC was used in tandem with a ThermoScientific ISQ EC mass spectrometer to analyze the samples. Once chromatograms were produced using a mass range of 80-200 *m/z* and a cone voltage of 10 in order to optimize the ionization of the molecules, extracted ion chromatograms were generated, which gave areas at each peak.

Results: Results using the MPIC method demonstrate reproducibility and linearity between runs. A working method validation in accordance with ANSI/ASB Standard 036 shows that the traditional external calibrator extractions agree with the MPIC method, with both techniques producing accurate results. Methamphetamine-spiked samples were quantified using both MPIC and traditional external calibration curves, and the respective concentrations are accurate and in agreement with each other.

When samples were spiked at 500 ng/mL, the MPIC procedure repeatedly predicted results within the \pm 20% range, with results ranging from 411 ng/mL to 563 ng/mL. To further validate this method, samples were then spiked at 120 ng/mL, and were predicted to have a concentration of 135 ng/mL. Traditional extraction procedures also predict results that fall within the acceptable \pm 20% range.

Conclusion/Discussion: MPIC results thus far demonstrate reproducible, accurate and comparable quantitative results to those obtained by the traditional external calibration method. This alternative technique will allow laboratories to add an isotopolgue calibration solution of different drugs of interest directly to case samples to obtain accurate quantifications, improving a laboratory's efficiency by decreasing TAT and therefore saving both time and money.

P71 - "When life gives you norsertraline, make de-amino-norsertraline!"

Madeleine E. Wood*, Sarah E. Boyle, Luette S. Muir, Joshua Z. Seither, Erin L. Karschner, and Jeffrey P. Walterscheid. Division of Forensic Toxicology, Armed Forces Medical Examiner System, 115 Purple Heart Dr., Dover AFB, DE 19902.

Background/Introduction: Selective serotonin re-uptake inhibitors (SSRIs) are among the most commonly prescribed antidepressant medications. Sertraline (Zoloft^{*}) is widely prescribed to treat depression, anxiety, and post-traumatic stress disorder. While sertraline itself is relatively safe, the necessity to measure it and its metabolite norsertraline in forensic cases may be more significant when considering interactions with other psychiatric medications or questioning compliance with prescribed therapy. However, the inherent structure of norsertraline is susceptible to spontaneous in-source fragmentation through loss of its amine group under standard electrospray ionization conditions. The protonated precursor ion often yields poor sensitivity, unreliable transition ratios, and erratic correlation between calibration and quality control values. This presentation reveals a subtle and unique method to solve issues associated with norsertraline confirmation and quantitation.

Objectives: This presentation demonstrates an alternative procedure for reliably identifying and quantifying norsertraline using liquid chromatography tandem mass spectrometry (LC-MS/MS).

Methods: High resolution MS/MS spectral data was obtained for both the intact (M+H)⁺ molecular ion and the deaminated ion using liquid chromatography quadrupole time of flight mass spectrometry (LC-QTOF-MS) on a Shimadzu Nexera X2 with Sciex X500R QTOF MS/MS. Based on high resolution MS/MS spectral data, transitions that used both molecular and deaminated ions as the precursor were selected and further optimized. Optimized compound dependent parameters included collision energy, declustering potential, entrance potential, and cell exit potential. Source parameters were optimized to improve the stability of transitions that used the amine-loss radical as the precursor ion. The LC gradient achieved baseline separation between norsertraline and sertraline. The fully validated method used solid phase extraction followed by LC-MS/MS analysis on a Shimadzu Nexera X2 UPLC with Sciex 4500 QTRAP MS/MS to quantify norsertraline and sertraline in blood and confirm in urine.

Results: A review of previous casework from January 2019 to present revealed consistent transition ratio failures at the lower end of the calibration range. In many cases, this resulted in the lowest calibrator being removed, which increased the limit of detection for that batch and in some instances, required specimens to be re-extracted. This highlighted the need to improve the analytical method. Further studies by LC-QTOF-MS revealed a prominent 275 m/z ion corresponding to deaminated norsertraline, instead of the anticipated 292.1 m/z protonated molecular ion. Both precursor ions were monitored throughout the entirety of development and validation using the MRM transitions 275>158.9, 275>129, 292.1>159, and 292.1>129.1. The protonated molecular ion (298.1>159, 298.1>135.1) and its deaminated ion (281>159, 281>135.1) were also monitored for the internal standard, norsertraline-¹³C₆. Substituting the amine loss as the precursor ion for both analyte and internal standard significantly improved the accuracy, sensitivity, and transition ratio stability of norsertraline. The fully validated method reliably detected 10 ng/mL of sertraline and norsertraline in both human blood and urine matrices. In addition, the method accurately quantified sertraline and norsertraline using a calibration model ranging from 10 to 500 ng/mL in blood with the r² value >0.99 and overall precision and accuracy less than 10.4% and 3.3%, respectively. A data comparison of the protonated molecular ion and deaminated ion will be presented.

Conclusions/Discussion: With sertraline being a commonly prescribed drug for depression, anxiety, and post-traumatic stress disorders, the need to fully characterize sertraline and its metabolite norsertraline are well within the scope of forensic toxicology. These results demonstrate the importance of accurately detecting these analytes across a wide concentration range while keeping turnaround time and costs low by avoiding unnecessary repeat testing and sample consumption. Implementation of these efficiencies improves the laboratory's ability to provide results in a timely manner to forensic pathologists and other investigative stakeholders, and thus maintain mission readiness of service members.

P72 - Quantification of phosphatidylethanol in whole blood and dried blood spots by LC-MSMS

Elizabeth Yulianto¹, Ariadna Capasso², Ralph DiClemente², Anait Marianian³, Darya Markova³, **Marta Concheiro**^{1,*},¹ John Jay College of Criminal Justice, City University of New York, NY; ² School of Global Public Health, New York University, NY; ³ Scientific Centre for Family Health and Human Reproduction Problems, Irkutsk, Russia.

Background/Introduction: Analyzing biomarkers of alcohol consumption has become useful in assessing drinking history in forensic and clinical settings. Phosphatidylethanol (PEth), formed by enzymatic reaction between ethanol and phosphatidylcholine, is one such alcohol biomarker. In comparison to the window of detection in blood using direct ethanol measurement (<8 h) and the ethanol minor metabolites, ethylglucuronide (EtG) and ethylsulfate (EtS) (<18 h), PEth has a detection time of 7 days or even up to one month, depending on the level of consumption. The long detection time makes PEth analysis useful in situations where direct ethanol, EtG or EtS measurement would not provide relevant information. For accurate and reliable measurement of PEth, monitoring the two most abundant analogs, PEth 16:0/18:1 and PEth 16:0/18:2, is recommended.

Objectives: Develop and validate a sensitive and specific method to quantify PEth 16:0/18:1 and 16:0/18:2 in whole blood and dried blood spots (DBS) by liquid chromatography tandem mass spectrometry (LC-MS/MS). Analyze and report PEth concentrations in 149 blood samples from pregnant women to detect possibility of prenatal alcohol exposure.

Methods: Whole blood was spiked with PEth 16:0/18:1 and 16:0/18:2 at concentrations of 20, 30, 75, 188, 375, 750 and 1500 ng/mL. Twenty μ L blood was spotted on Whatman 903 Protein Saver Cards (Fisher Scientific) and allowed to dry overnight. For PEth extraction, 50 μ L whole blood samples or 12.7 mm whole punches from DBS were extracted by sonication in methanol, followed by centrifugation, evaporation, and reconstitution with 100 μ L solvent mixture (water/2-propanol/acetonitrile/formic acid, 34/50/15/0.05). Sonication was done for 20 min for whole blood and 60 min for DBS. The chromatographic separation was performed in gradient mode with a reversed phase C5 column (2 x 50 mm, 5 μ m). Mobile Phase A was 30/70 water/acetonitrile with 0.6 mM Ammonium Acetate, and mobile Phase B was 100% 2-propanol. We employed electrospray ionization (ESI) in negative mode, and 2 MRM transitions were monitored per analyte.

Results: 7-point calibration curve with concentrations ranging from 20 to 1500 ng/mL (n=5) was linear with a correlation coefficient >0.994 (not forced, $1/x^2$ weighting), and the limit of quantification (LOQ) was 20 ng/mL in both matrices. Process efficiencies at the low (57 ng/mL), medium (200 ng/mL) and high (1180 ng/mL) QCs were >47% for whole blood samples and >100% for DBS samples. In whole blood, bias ranged from -10.5 to 6.8%, intra-day imprecision was <16.2% and inter-day imprecision was <12.6% at the 3 QC levels (n=15; 3 replicates, 5 days). In DBS, bias was from -12.2 to -0.5%, intra-day imprecision was <17.8% and inter-day imprecision was <14.8% (n=15; 3 replicates, 5 days). 149 authentic cases were tested, and 6 cases were found to be positive for PEths. Two samples were positive for PEth 16:0/18:1 only, and these were both at the LOQ. A third case was positive for PEth 16:0/18:1 at the LOQ, and positive for PEth 16:0/18:2 at 24.6 ng/ mL. The remaining three positive cases were in the range of 23.3-120.6 ng/mL for PEth 16:0/18:1 and 23.5-150.8 ng/mL for PEth 16:0/18:2. The cases positive for both PEths had similar concentrations of each.

Conclusion/Discussion: A sensitive and specific method was developed in whole blood and DBS for PEth 16:0/18:1 and PEth 16:0/18:2 with a 10 min run time and a LOQ at 20 ng/mL. Smaller samples of blood in DBS (20 μ L) are required than in whole blood (50 μ L) for similar results.

P73 - Analytical method for the identification and quantitation of 21 cannabinoids in neat oral fluid by UHPLCMS/MS

Martin Jacques*, Michael Clark, Melissa Beals, Brian Dorsey, David Kuntz; Clinical Reference Laboratory, Lenexa, KS.

Background/Introduction: Testing for cannabinoid use has been driven by concerns about consumer health, unregulated product content, and workplace safety. The use of oral fluids has been accepted by the US Department of Health and Human Services as a matrix for the Federal Drug Testing Program and allows for noninvasive sample collection. The method developed by our laboratory provides an evaluation of 21 cannabinoids in oral fluid at concentrations from 0.02510 ng/mL.

Objectives: Develop an analytical method for extraction, detection, and quantitation of () Δ 9-Tetrahydrocannabinol, Δ 9CarboxyTetrahydrocannabinol, 11-Hydroxy- Δ 9-Tetrahydrocannabinol, 8 β Hydroxy- Δ 9-Tetrahydrocannabinol, Δ 9-Tetrahydrocannabivarin, Δ 9CarboxyTetrahydrocannabivarin, (-)- Δ 8- Tetrahydrocannabinol, Δ 8-Carboxy-Tetrahydrocannabiol, Δ 8-Tetrahydrocannabivarin, Cannabidiol, 7Hydroxy-Cannabidiol, 7Carboxy-Cannabidiol, Cannabidiolic Acid, Cannabinol, Cannabichromene, Cannabichromenic Acid, Cannabigerol, Cannabigerolic Acid, Cannabicyclol, and Cannabicyclolic Acid in oral fluid by liquid chromatography-tandem mass spectrometry (LC-MS/MS) for a controlled dosing research study.

Methods: Synthetic oral fluid fortified with 21 cannabinoids at known concentrations was analyzed to establish linearity and evaluate assay interference and matrix effects. A liquid-liquid extraction was performed using a 0.500-mL sample aliquot with 0.1M Ammonium Bicarbonate (pH 10.5), tert-butyl methyl ether, and isopropanol, followed by drying and reconstitution with 50:50 0.1% Acetic Acid in DI H₂O: Acetonitrile. Separation was performed by a Shimadzu Nexera LC30AD HPLC system equipped with a Phenomenex Kinetex C18 column. Aqueous mobile phase-A, 0.1% Acetic Acid in water, and organic mobile phase-B, 50:50 Acetonitrile: Methanol, combined in a gradient over the 16.00 minute run at a 0.750 mL/ min flow rate. MS-MS analysis was performed by a Sciex API7500 tandem mass spectrometer using electrospray ionization in both positive and negative MRM modes.

Results: Linearity was established using single-point calibration through analysis of replicates at 13 concentrations ranging from 25 pg/mL to 10 ng/mL. All replicate values were within $\pm 20\%$ of target. At the cutoff concentration (2.0 ng/mL) and 40% of cutoff (0.8 ng/mL low control), accuracy was within $\pm 10\%$ of target and CV was <10% for all analytes. Limit of Detection/Quantitation was determined to be 50 pg/mL for 8 β -OH- Δ 9-THC and 25 pg/mL for all other analytes. The upper limit of linearity was confirmed at 5 or 10 ng/mL, analyte dependent, where replicates were within $\pm 20\%$ of target, internal standard abundances were within 50% of the calibrator, and a negative control injected following the final replicate contained <10% of the target analyte by ion abundance compared to the calibrator. Interference and matrix effect studies did not reveal issues with identification, quantitation, or ion suppression. Extraction recovery experiments did not indicate significant analyte loss through liquid-liquid extraction. In addition to the low control, a high control was designated at 125% of cutoff (2.5 ng/mL). A conversion control containing 5.0 ng/mL of Cannabidiol, 7-Hydroxy-Cannabidiol, 7-Carboxy-Cannabidiol, and Cannabidiolic Acid was included to monitor the potential conversion of Cannabidiol and its metabolites to Δ 9- and Δ 8-Tetrahydrocannabinol and corresponding metabolites.

Conclusion/Discussion: Designed for a research study using oral fluid, the alkaline sample extraction favored recovery of parent drug compounds; with the utilization of polarity switching, the sensitivity of the API7500 MS/MS was able to offset reduced recovery of acidic metabolites. For evaluation of the cannabinoid elimination phase, the instrument method was optimized for low-level analyte detection; samples with concentrations greater than the linear range were reanalyzed with a diluted preparation.

The analytical method reliably identified and quantitated 21 cannabinoids in oral fluid in low pg/mL levels, adding to scientific knowledge of cannabinoid metabolism and distribution in oral fluid. This method demonstrated selectivity, accuracy, and reproducibility for federally-sponsored research studies.

P74 - An expansion validation of a combined cocaine-opioids method by LC-MS/MS

Megan Savage*, Lindsay Glicksberg, Brittany K. Casey. Dallas County Southwestern Institute of Forensic Sciences, Dallas, TX.

Background/Introduction: In 2016, the Dallas County Southwestern Institute of Forensic Sciences (SWIFS) Toxicology Section (the Laboratory) developed and validated a method for the quantitation of cocaine, opiates, and metabolites by LC-MS/MS. This validation served multiple purposes: decrease the lower limit of quantitation/detection and overall analytical run time, and consolidate analytes from three separate quantitation methods using GC-MS or GC-FID/FID. The Laboratory chose to re-validate this method to incorporate additional opioids, related analytes of interest, and metabolites. This expansion validation transitioned additional analytes from the comprehensive drug quantitation analysis via GC-FID/FID to LC-MS/MS, enabled quantitation of analytes previously reported qualitatively, and satisfied ANSI/ASB Scope Standards 119, 120, and 121.

Objectives: To expand and re-validate the Laboratory's Cocaine, Opiates, and Metabolites by LC-MS/MS quantitative method.

Methods: The validation was conducted according to the ANSI/ASB Standard 036: Standard Practices for Method Validation in Forensic Toxicology. Excepting the addition of a sixth calibrator point within the established analytical ranges, the previously validated solid phase extraction method and instrument parameters (LC gradient, flow rate, mobile phases, temperature) did not change with the addition of the new analytes. The new analytes were optimized and retention times were established. The new analytes were added to one of the existing concentration ranges in this assay, with the exception of gabapentin (Table 1), for which a new concentration range was established. Each analyte, except loperamide, is quantitated against its own corresponding isotopically labeled internal standard.

Table 1 Analytes in the Cocaine, Opiates, and Metabolites by LC-MS/MS method and their corresponding analytical ranges.

Linear Range (ng/mL):	1-100	5-500	10-1000	50-5,000	200-20,000
Analytes	Oxymorphone	6-MAM	EME	BE	Gabapentin*
	Hydromorphone	Loperamide*	Morphine		
	Naloxone		Dihydrocodeine		
	Norbuprenorphine		Codeine		
	Acetyl fentanyl		Oxycodone		
	Fentanyl		Hydrocodone		
	Buprenorphine		Cocaine		
	Norfentanyl*		CE		
			Methadone		
			Mitragynine*		
			Demethylmeperidine*		
			Meperidine*		
			Pentazocine*		
			Tramadol*		
			O-desmethyltramadol*		
			EDDP*		
Low, Mid-Range, High	3	15	30	150	600
QC (ng/mL) 	50	250	500	2,500	10,000
	80	400	800	4,000	16,000

^{*}new analyte

6-MAM: 6-monoacetylmorphine, EME: ecgonine methyl ester, CE: cocaethylene, BE: benzoylecgonine

The validation parameters assessed included calibration model, bias, precision, matrix effects, interferences, dilution integrity, limit of quantitation/detection, carryover, sample extract stability, and chromatographic stability (RT, ion ratios).

Results: All analytes eluted within the previously validated LC program with acceptable peak shape, retention time stability, and ion ratio stability. Calibration models were determined to either be linear, weighted (1/x) or quadratic, weighted (1/x) for all analytes. Analytes with a quadratic curve fit were further evaluated for heteroscedasticity using residual plots. The bias and precision for all analytes at the low, medium, and high QC concentrations met the ANSI/ASB Standard 036 requirement of $\leq \pm 20\%$ of the target concentration (bias) and $\leq 20\%$ (precision, %CV). Matrix effect was evaluated for the analytes, at the low and high concentration, and for all internal standards. Ion suppression or enhancement (ISE) was observed for select analytes and internal standards... To ensure that ISE did not impact the limit of detection/quantitation, the lowest calibrator was evaluated in a minimum of nine different blood sources; no impact was observed. No interferences were observed from the presence of over 120 commonly encountered analytes. Additionally, no carryover was observed following the injection of a sample spiked at a concentration greater than the highest calibrator, and samples were deemed stable for next day injections (<20% loss). A 10x dilution was suitable for analytes (<20% difference from undiluted specimen).

Conclusion/Discussion: The Cocaine, Opiates, and Metabolites by LC-MS/MS method at SWIFS was expanded to include additional analytes and curve point. This updated method was successfully re-validated following ANSI/ASB Standard 036 for the quantitation of 24 analytes and qualitative identification of loperamide.

P75 - The development of a virtual liquid chromatography method development tool

Jamie York^a, John Garrett^b, Chris Nelson^a, Tim Yosca^a, Justin Steimling^a, and ***Mel Urich^a**. ^aRestek, Bellefonte, PA. ^bAnalytical Innovations, Inc., Dayton, OH.

Background/Introduction: The development and optimization of Liquid Chromatography (LC) separations can be time consuming and costly, often requiring a number of steps including literature research, column selection, method scouting, method development, and method optimization. In an effort to eliminate these steps, an instrument-free, software modeling tool that gives users the ability to select compounds from a database and instantly model a separation on different column phases was developed. Optimization of the model can be performed while maintaining critical pair separations by adjusting for instrument/system effects (e.g. dwell volume and extra column volume), mobile phase preferences, number of gradient steps, and more. The modeler delivers a fast, no-cost starting point. The initial database consists of a Drugs of Abuse (DoA) library containing approximately 250 compounds with plans to continually expand the utility.

Objectives: To develop a chromatogram modeling tool that allows users to develop and optimize their LC methods virtually, improving data quality and laboratory efficiency without time-consuming in-lab method development.

Methods: To build the chromatogram modeler, a DoA library containing approximately 250 compounds was created. Retention times were first collected using a fast/slow gradient, 30°C/60°C temperature points, and ACN/MeOH mobile phases on a single column dimension. Some additional data outside of these runs were also collected for the development of a semi-empirical correction factor that was used to improve modeling accuracy.

To assess the accuracy of the modeler, experiments comparing compound retention time values between wet-lab and modeled data were conducted. After the initial DoA library was built, the modeler was evaluated over four increasingly more complex stages of verification. In the final, most complex stage, new compounds not previously part of the initial DoA library were added and then compared by testing two different column dimensions, two different columns lengths, two different mobile phases, two different stationary phases, three different gradients programs, and three different temperatures against modeled retention time values. Because the semi-empirical correction factor was developed using only the original library compounds, this stage assessed the viability of adding future compounds to existing libraries.

Results: An online chromatogram modeling tool was successfully developed that allows users to select columns and compounds for separation. A modeled chromatogram and instrument-ready conditions are automatically generated and can be further optimized by users (Figure 1). During software development, the acceptance criteria for retention time agreement between wet-lab and modeled values was set at +/- 15 seconds. This range was chosen because it represents a typical MRM window. In the most complex portion of the verification, 704 retention time data points were collected in total for the 25 compounds used in the evaluation. Only 13 data points exceeded the +/- 15 second window with no compounds missing acceptance criteria by more than five seconds. The overall pass rate was 98.2%.



Figure 1: Chromatogram Modeler Software Interface

Conclusion/Discussion: For LC method developers, novice and expert, who either lack the expertise, or the time, to develop separations quickly and accurately, this free tool can be used to deliver a fast, no-cost starting point for method development and optimization. This novel, virtual method development software can improve turnaround time, increase throughput to existing methods, and offer an on-demand consultative user experience.

P76 - Identification and quantitation of 13 cannabinoids in whole blood by UHPLC-MS/MS

Melissa Beals*, Martin Jacques, Michael Clark, David Kuntz; Clinical Reference Laboratory, Lenexa, KS.

Background/Introduction: Since the legalization of hemp in 2018, several of the more than 120 identified phytocannabinoids have become popular for recreational and naturopathic medical use. Detection and quantitation of these compounds is important for safety and compliance as their use is now widespread. The method developed by our laboratory provides a detailed analysis of whole blood specimens, evaluating the presence of 13 cannabinoids including Cannabidiol (CBD) and CBD metabolites at concentrations from 0.200150 ng/mL.

Objectives: Develop an analytical method for the extraction, detection, and quantitation of Cannabidiol, Cannabinol, $(-)-\Delta 9$ -THC, $(-)-\Delta 8$ -THC, 7-Carboxy-Cannabidiol, 7-Hydroxy-Cannabidiol, 11-Hydroxy- $\Delta 9$ THC, $\Delta 8$ Carboxy-THC, $\Delta 9$ Carboxy-THC, Cannabigerol, Cannabidiolic Acid, Cannabicyclol, and Cannabichromene in whole blood by LC-MS/MS for a controlled dosing research study.

Methods: Whole blood standard solutions containing 13 cannabinoids at known concentrations were analyzed to establish linearity, investigate assay interference, and evaluate matrix effects. Samples were prepared by mixing a 0.400 mL aliquot of whole blood sample with internal standard solution and cold 0.1% Formic Acid in Acetonitrile, adding 0.1% Formic Acid in DI H₂O, and loading the solution onto an Agilent Captiva EMR—Lipid 3 mL Cartridge in a silanized glass culture tube. Following sample elution, the cartridge was rinsed with 80:20 Acetonitrile: DI H₂O and eluted into the same tube. A liquid-liquid extraction was then performed using the combined eluent and 2:1 Hexanes: Ethyl Acetate; the organic components were subsequently dried and reconstituted with 0.1% Formic Acid in 50:50 DI H₂O: Methanol. Separation was performed by a Shimadzu Nexera LC40D X3 HPLC system utilizing a WatersTM CORTECS C18+ column and aqueous mobile phase (A), 0.1% Acetic Acid in water, and organic mobile phase (B), 0.1% Acetic Acid in Acetonitrile at a flow rate of 0.5 mL/minute. MS-MS analysis was conducted by a Sciex API7500 tandem mass spectrometer using electrospray ionization in both positive and negative MRM modes.

Positive Ionization:						
Analuta		RT				
Analyte	Precursor	Quantifier	Qualifier	(mins)		
CBD	315.2	193.1	235.1	8.16		
CBN	311.2	208.0	223.0	9.56		
Δ9-ΤΗϹ	315.2	193.1	123.0	10.50		
Δ8-THC	315.2	193.1	93.1	10.75		
Negative Ionization:						
7-COOH-CBD	343.1	297.1	231.1	3.31		
7-OH-CBD	329.2	299.2	268.1	3.51		
11-OH-THC	329.2	173.0	268.1	5.54		
Δ8-COOH-THC	343.1	245.1	191.0	5.62		
Δ9-СООН-ТНС	343.1	191.0	245.1	5.83		
CBG	315.0	191.1	136.0	8.13		
CBDA	357.2	245.1	179.0	8.32		
CBL	313.2	191.0	203.0	11.43		
СВС	313.2	191.0	203.0	11.90		

Results: Accuracy and precision of 5 replicates at 14 concentrations from 100 pg/mL to 150 ng/mL using a 5.0 ng/mL single-point cutoff calibrator were used to determine assay linear range. Linearity was established from 0.20 ng/mL to 50 ng/mL for Cannabidiol and Cannabidiolic Acid, from 0.50 ng/mL to 150 ng/mL for (-)- Δ 9-THC, Cannabigerol, Cannabicy-clol, and Cannabichromene, and from 0.20 ng/mL to 150 ng/mL for all other analytes; no carryover was observed at the highest concentrations. CV was <10% for all analytes and replicates at each concentration were within ±20% of target, with the exception of one value for Cannabigerol, which had no labeled internal standard. Interference and matrix effect 176

studies used negative and spiked samples at 40% of cutoff concentration. Matrix effect was evaluated for 10 different donor samples, and interference was investigated with 119 compounds, including over-the-counter, illicit, and commonly prescribed drugs. Neither study revealed issues with identification, quantitation, or ion suppression.

Conclusion/Discussion: The analytical method reliably identified and quantitated 13 cannabinoids in whole blood at pg/ mL levels, contributing to the scientific knowledge of cannabinoid metabolism and distribution in whole blood. This method demonstrated selectivity, accuracy, and reproducibility for federally-sponsored research studies.

P77 Analytical method for the determination of 15 cannabinoids in urine by UHPLC-MS/MS

Michael Clark*, Martin Jacques, Zayne Williams, Melissa Beals, David Kuntz; Clinical Reference Laboratory, Lenexa, KS.

Background/Introduction: Cannabinoid use has increased significantly subsequent to the ratification of the 2018 United States Farm Bill. A rugged analytical method for the identification and quantitation of cannabinoids is valuable in determining recreational and medical use of these compounds, as well as monitoring potential contaminations in over-the-counter cannabinoid products. The method developed by our laboratory allows for the determination of 15 different cannabinoids in urine.

Objectives: Develop an analytical method for the extraction, detection, and quantitation of (-)-Δ9-THC, Δ9CarboxyTHC, 11-Hydroxy-Δ9-THC, Δ9-Tetrahydrocannabivarin, Δ9Carboxy-Tetrahydrocannabivarin, (-)-Δ8-THC, Δ8-Carboxy-THC, Cannabidiol, 7Hydroxy-Cannabidiol, 7-Carboxy-Cannabidiol, Cannabidiolic Acid, Cannabinol, Cannabichromene, Cannabigerol, and Cannabicyclol in urine by LC-MS/MS for a controlled dosing research study. This method was validated in accordance with NLCP Mass Spectrometry Guidelines.

Methods: Normal human urine fortified with bovine serum albumin was spiked with 15 cannabinoids at known concentrations and analyzed to establish linearity and evaluate assay interference and matrix effects. Sample preparation involved dual hydrolysis of a 0.500 mL aliquot of urine specimen using BG Turbo β-glucuronidase/0.1M phosphate buffer (pH 6.8) solution followed by the addition of 5N Potassium Hydroxide. Samples were neutralized with 5N Formic Acid and the mixture was eluted through an Agilent Captiva EMR—Lipid 3 mL Cartridge in a silanized glass tube. The cartridge was then rinsed with 80:20 Acetonitrile: DI H₂O and eluted into the same tube. A liquid-liquid extraction was performed using the eluent, pH 4.8 0.4M Ammonium Acetate buffer, and 2:1 Hexanes: Ethyl Acetate. The organic components were decanted, dried, and then reconstituted with 0.1% Formic Acid in 50:50 DI H₂O: Methanol. Analysis was executed by a Shimadzu Nexera LC40D X3 UHPLC equipped with a WatersTM CORTECS C18+ column coupled to a Sciex API7500 tandem mass spectrometer. The aqueous mobile phase (A), 0.1% Acetic Acid in water, and organic mobile phase (B), 0.1% Acetic Acid in Acetonitrile, flowed at a consistent rate of 0.5 mL/minute over the 15-minute run time. MS-MS analysis was conducted using electrospray ionization in both positive and negative MRM modes.

HPLC Gradient					
Time (min)	Pump A%	Pump B%			
0.50	53.0	47.0			
1.50	52.0	48.0			
2.50	44.0	56.0			
4.50	44.0	56.0			
5.50	37.5	62.5			
9.50	27.5	72.5			
12.00	27.5	72.5			
12.50	15.0	85.0			
13.50	15.0	85.0			
13.51	53.0	47.0			
14.51	53.0	47.0			
15.00	STOP				

Negative Ionization						
Ameliate	Transitions				LOD/LOQ (ng/mL)	ULOL (ng/mL)
Analyte	Precursor Quantifier Qualifier	KI (MINS)				
7-COOH-CBD	343.2	179.0	231.1	3.51	0.50	100.0
Δ9-СООН-ТНСV	315.2	217.1	163.1	3.78	0.50	100.0
Δ8-СООН-ТНС	343.1	245.1	191.2	5.90	0.50	100.0
Δ9-СООН-ТНС	343.1	245.2	191.1	6.13	0.50	100.0
CBDA	357.2	245.1	227.1	9.02	0.50	100.0
7-OH-CBD	329.2	268.1	179.0	3.72	0.50	100.0
CBG	315.0	136.0	177.2	8.84	0.50	50.0
Positive Ionization						
11-OH-THC	331.2	193.1	201.1	5.83	0.50	100.0
THCV	287.3	135.0	123.0	8.53	1.00	100.0
CBD	315.2	193.2	135.2	8.86	0.50	100.0
CBN	311.1	241.0	208.0	10.40	0.50	100.0
Δ9-ΤΗϹ	315.1	193.2	123.0	11.38	0.50	100.0
Δ8-ΤΗϹ	315.1	193.2	135.3	11.65	0.50	50.0
CBL	315.2	193.0	123.0	12.36	2.00	100.0
CBC	315.2	193.1	123.1	12.91	2.00	100.0

Results: Linearity was established for all analytes and no carryover was observed at the highest concentrations. Interference and matrix effect studies did not reveal any issues with identification, quantitation, or ion suppression.

Conclusion/Discussion: The analytical method reliably identified and quantitated 15 cannabinoids in urine at pg/mL levels, contributing to the scientific knowledge of cannabinoid metabolism and distribution in urine. This method demonstrated

P78 - In vitro mitragynine stability

Nancy Kedzierski, M.S.*, Dani Mata, M.S., D-ABFT-FT

Orange County Crime Laboratory, Santa Ana, CA.

Background/Introduction: Mitragynine is one of the primary psychoactive alkaloids found in the Kratom products from the *Mitragyna speciosa*. The drug exhibits dose dependent effects in that it can act as a stimulant at low doses and as a narcotic analgesic at higher doses. Mitragynine is a drug of interest in both driving under the influence of drugs (DUID) and postmortem investigations due to its contribution to both impaired driving symptomology and as a contributing factor in the cause of death. Drug stability is an important factor to be considered in DUID and postmortem investigations due to the high turnaround times in many laboratories. The limited studies conducted to date on mitragynine stability have shown a significant decrease in mitragynine concentrations after 30 days in antemortem blood regardless of storage conditions. The drug loss appears to occur more rapidly under strong alkaline or acidic conditions.

Objectives: The objective of this study was to evaluate the stability of mitragynine in various matrices under different sample handling conditions. Antemortem blood, postmortem central blood, urine, brain, and liver were fortified with mitragynine and evaluated over ~12 months under single and multi-use aliquots.

Methods: Pooled negative samples were spiked with mitragynine to be on the upper end of the quantitative calibration range approximately 300 ng/mL. The various matrices were then tested as either single-use aliquots that were discarded after analysis or in a pooled manner that were brought to room temperature before analysis and then placed back in either refrigeration or frozen conditions until subsequent testing. Samples were tested at time 0, once a week for the first month, once every other week for the next two months, once a month out to 6 months, and once more a year later for non-tissue samples and the data was plotted as an average of 3 replicates for each time point. Quantitation occurred using a validated liquid chromatography tandem mass spectrometer with a limit of quantitation at 10 ng/mL. Samples were monitored using a decrease of 20% from the initial concentration as a stability cut-off point. Brain tissue was studied although not validated for quantitative purposes upon initial validation due to unacceptable bias data.

Matrix	Storage condition	рН	Storage condition	Time to 20% loss [Days]
AM Blood	4°C	7.6	Single-use	82
			Multi-use	82
PM Blood	4°C	7.4-7.6	Single-use	13, 40
			Multi-use	13
Urine	4°C	6.2-6.4	Single-use	112
			Multi-use	82
Liver	-20°C	6.0	Single-use	112
			Multi-use	82
Brain*	-20°C	4.8	Single-use	<6
			Multi-use	<6, 82

Results:

The data showed a slight benefit to single-use aliquots for mitragynine stability but overall, the rate of mitragynine loss within each sample type was mostly paralleled. In 2 instances, PM blood single-use and brain multi-use, the mitragynine concentration dropped below 20% of the initial concentration, was back in range, then fell below at a second date.

Conclusion/Discussion: Mitragynine does appear to have a relatively short stability time frame in various fortified matrices and under various sample handling conditions. While detection of the drug's presence is still beneficial in DUID and postmortem investigations, any correlations to the concentration found in a sample should be considered carefully with a longer collection to testing interval. Further work should be conducted to see if there is any difference in mitragynine stability in authentic samples.
P79 - Toxicology screening of human blood using quadrupole-time of flight (QTOF) mass spectrometry

Evelyn H. Wang¹, Sarah R. Olive¹, Neil J. Loftus², Jennifer C. Davis¹, **Rachel A. Lieberman**^{*1}, Christopher T. Gilles^{1.,1}Shimadzu Scientific Instruments, Columbia, MD, ²Shimadzu Corporation MSBU, Manchester, United Kingdom.

Background/Introduction: Initial toxicology screening is performed on all toxicology samples to screen for the presence of certain drug classes and compounds. Traditionally, toxicology screening has been done using immunoassay which is limited to specific drug classes and can result in false positives. With the addition of many new novel psychoactive substances and unknown compounds, the demand to identify these unknown compounds has increased. To meet the increasing demand for a more rapid and effective toxicology screening method, a high-resolution accurate mass quadrupole-time of flight (Q-TOF) mass spectrometer with a comprehensive library was used to develop a screening workflow. A method was developed on a Q-TOF to screen toxicologically significant compounds in blood extracts.

Objectives: This method focuses on the use of a high-resolution mass spectrometer for screening blood samples for common abused drugs using data independent analysis and library matching.

Methods: Solid phase extracted blood samples were spiked with a panel of commonly abused drugs (benzoylecgonine, codeine, fentanyl, hydrocodone, hydromorphone, methadone, methamphetamine, morphine, and temazepam) at concentrations ranging from 5-5000 ng/mL. All components were separated using a Shimpack Velox column (2.1 x 100 mm; 2.7µm) with a mobile phase of water and methanol with 2 mM ammonium formate and 0.002% formic acid. Data was acquired using a MS TOF scan event and DIA-MS/MS in positive ion mode. MS scan range was m/z 40-900 and each DIA-MS/MS mass scan had a variable precursor isolation width and a collision energy spread of 5-55 V. The acquired data allows for simultaneous highly specific targeted quantitation and non-targeted screening with library verification.

Results: A panel of commonly abused drugs (benzoylecgonine, codeine, fentanyl, hydrocodone, hydromorphone, methadone, methamphetamine, morphine, and temazepam) was used to develop a routine toxicology screening workflow by high resolution LC-MS/MS. For targeted workflows, the method included a toxicologically relevant compound database with a predefined retention time and MS/MS fragmentation energy for over 900 compounds. Each accurate mass product ion spectrum in the compound database was acquired using targeted MS/MS and a precursor ion isolation width of 1 Da.

Using the test panel, all compounds were successfully detected and positively identified in each calibration standard 5, 50, 500, and 5000 ng/mL. Spiked toxicology compounds amphetamine (m/z 136.1110; RT=3.47 min), benzoylecgonine (m/z 290.1384; RT=4.71 min), cocaine (m/z 304.1547; RT=5.15 min), gabapentin (m/z 172.1327; RT=3.28 min), alprazolam (m/z 309.0903; RT=8.71 min), fluoxetine (m/z 310.1420; RT=8.712 min), methamphetamine (m/z 150.1283; RT=3.70 min), and morphine (m/z 286.1450; RT=3.17 min) were detected in the blood samples. Highly confident compound identification was reported for all targets with similarity scores higher than 80 using library search paraments weighted for both mass accuracy and ion signal intensity with limited retention time windows.

Conclusion/Discussion: The toxicology workflow was optimized for both targeted LC-MS/MS analysis and untargeted toxicology screening to detect and identify a range of targets including illicit drugs, adulterants, unregulated supplements, and prescription medications. To increase reporting confidence in compound identification for targeted analysis the accurate mass, isotopic distribution, accurate mass MS/MS library verification on the product ion spectrum, and retention time (RT) were used to target large panels of compounds of interest. In this method, the chromatographic separation was optimized for a diverse chemical space. Method parameters for peak integration and spectrum processing considered both trace level and component saturation to consider likely toxicology workflows. This workflow demonstrated highly confident reporting in routing toxicology screening for over 900 compounds using a QTOF mass spectrometer.

P80 - Quantitation of 9 opioids by an automated and fully integrated dried matrix spot module and UHPLC-MS/MS system

Richard Gibson*, Stephanie Samra, Jingshu Guo, Kristine Van Natta, Courtney Patterson. Thermo Fisher Scientific, San Jose, CA.

Background/Introduction: The United States is facing an opioid crisis with 80,816 reported deaths by overdose in 2021. Opioids severely impact cognitive abilities, causing societal dangers such as driving under the influence. This highlights the importance of being able to easily collect samples and sensitively detect common opioids.

Mass spectrometry (MS) is increasingly used in forensic toxicology to quantify analytes from whole blood as it can offer higher sensitivity and selectivity compared with other analytical techniques. Testing blood that has been spotted onto dried matrix spot (DMS) cards has advantages since it simplifies sample collection and analysis. Collection of whole blood spots on cards is quick, minimally invasive, and enables easy sample storage.

Automated DMS modules may utilize flow through desorption technology to perform the on-line and efficient extraction of analytes in spotted and dried whole blood and may be coupled with UHPLC-MS/MS systems for highly sensitive analysis. Here we demonstrate the successful coupling of a combined DMS module and UHPLC system with a triple-stage quadrupole mass spectrometer for the sensitive and robust detection and quantitation of a representative opioid panel in dried blood spots within one fully automated and integrated workflow. The panel consists of 6-acetylmorphine, co-deine, fentanyl, hydrocodone, methadone, morphine, O-desmethyltramadol, oxycodone, and tramadol.

Objectives: Demonstrate automated extraction and analysis of dried blood spot cards for a representative opioid panel in whole blood for forensic toxicology.

Methods: Non-labeled standards were combined into a stock solution, diluted in methanol and prepared in whole blood to create a 15-point calibration curve ranging from 0.05 to 400 ng/mL. Quality control (QC) standards were prepared at a concentration of 1.0, 5.0, and 50 ng/mL in whole blood. Ten microliters of each calibrant were spotted centrally on a HemaXis cassette card with preprinted circles and left to dry overnight. All samples were spotted in triplicate. A 10 ng/mL internal standard (IS) working solution was created by combining deuterated and carbon-13 isotopically labeled standards.

Dried cards were loaded onto a Thermo Scientific[™] Transcend[™] DSX-1 system that utilized flow-through desorption technology to extract the contents of the spiked dried blood spot and add 20 mL of IS mix to each eluant. TurboFlow sample clean-up was then performed with a TurboFlow[™] Cyclone-P (0.5 x 50 mm) column and a mobile phase consisting of 0.1% formic acid in water. Analytical separation was performed on a biphenyl column (2.6 mm, 2.1 x 50 mm) maintained at 40 °C. Mobile phases consisted of 10 mM ammonium formate in water with 0.05% formic acid and 10 mM ammonium formate in methanol with 0.05% formic acid. Data was acquired using a Thermo Scientific[™] TSQ Altis[™] mass spectrometer with selected reaction monitoring scans collected for one quantifier and one qualifier ion for each opioid and corresponding isotopically labeled standard. The DSX-1 system was controlled by Thermo Scientific[™] Aria[™] MX software for data acquisition, and data processing was completed using the Thermo Scientific[™] TraceFinder[™] 5.1. The entire method took 9 minutes.

Results: All 9 opioids eluted chromatographically between 4.21 and 5.98 minutes with baseline separation of all isomers. Corresponding stable-isotope labeled standards were included for absolute quantitation of each opioid. The TSQ Altis™ demonstrated LOQs at or below 1.0 ng/mL for each of the 9 opioids, quantified linearly up to 400 ng/mL with an R² > 0.98.

Conclusion/Discussion: A sensitive 9-minute method for the detection and quantitation of 9 opioids in dried blood spots was developed using an automated dried matrix spot analyzer coupled to a triple quadrupole mass spectrometer for clinical toxicology purposes.

P81 - Applicability of a novel automation platform for column-based drug of abuse extraction prior to LC/MS or GC/MS analysis.

Russell Parry*, Alan Edgington, Lee Williams, Helen Lodder, Adam Senior, Charlotte Hayes, Geoff Davies, Claire Desbrow & Dan Menasco.¹Biotage GB Limited, Distribution Way, Dyffryn Business Park, Cardiff, CF82 7TS, UK.

Background/Introduction: Historically, silica-based solid phase extraction (SPE) columns have been used for drugs of abuse testing, with the exact choice being dependent on drug functionality. GC/MS sensitivity requirements traditionally dictated larger volume sampling resulting in the use of columns capable of accepting volumes up to 10mL. Reduced sample size has been achieved due to increased specificity using GC-MS/MS or the movement to LC/MS analysis.

Objectives: This poster will present a dedicated sample preparation platform capable of processing volumes associated with drugs of abuse testing whether using GC/MS or LC/MS end points. Emphasis on improved speed, workflow advantages compared to offline processing will be demonstrated along with method transfer and performance with respect to analyte recoveries, RSDs and calibration.

Methods: Individual analyte panels were extracted from urine for analysis using GC/MS while a larger multi-component target panel was analysed using LC-MS/MS. Previously developed extraction protocols were compared between offline positive pressure processing and automation using a Biotage[®] Extrahera[™] HV-5000 system. Sample extraction was performed using silica-based SPE chemistries. LC-MS/MS analysis was performed using a Shimadzu Nexera UHPLC system coupled to an 8060 triple quadrupole mass spectrometer. MRM transitions were selected using the most intense precursor ions. GC-MS analysis was performed using an Agilent 7890A GC coupled to a 5975C MSD. Positive ions were acquired using electron ionization operated in SIM mode for the respective derivatised precursor.

Results: Drug of abuse panels including amphetamines, benzodiazepines, cocaines, THC and opiates were investigated as a large panel for LC/MS or individual panels for GC/MS analysis. Previously developed extraction protocols were optimised for flow control and processing using traditional positive pressure processing manifolds. The extraction protocols were then transferred to the Extrahera™ HV-5000 sample preparation platform. Solvent and sample pipetting parameters, sample mixing and positive pressure processing to affect precise flow control were optimized for each panel and matrix. Extraction recoveries for all panels were typically greater than 80% using mixed-mode SPE in either 3mL or 6mL column formats. Supported liquid extraction demonstrated equally high analyte recoveries but was limited to a maximum loading volume of 1 mL matrix. Good method correlation was observed between manual positive pressure and automated processing for all analyte panels in terms of analyte recovery and overall signal, while the automation generally provided better precision and accuracy for both LC/MS and GC/MS testing. Calibration curves demonstrated excellent linearity and coefficients of determination, $r^2 > 0.99$ for all analyte in the respective panels covering prescribed guidelines and required lower limits of quantitation. Sample throughput was dependent on several factors: processing volumes, extraction sorbent geometry and resulting automation bed layout. 3 mL columns were processed in 48 position bed layout, 6 mL columns in 24 positions. When assay processing volumes were below column headspace capacity, the use of 3 mL columns doubled processing throughput compared to the 6 mL option. However, where volumes exceeded column capacity the use of 24/48 position was less clear in terms of throughput. Final format selection depended on assay complexity, throughput requirements and limit of quantitation.

Conclusion/Discussion: This poster demonstrates the effectiveness of the Extrahera^M HV-5000 for the automated extraction of drugs of abuse prior to LC/MS or GC/MS analysis. Automation throughput was drastically improved using 5000 μ L tips for assays using column formats and/or when using larger sample/solvent processing volumes. Full results, discussion and conclusion will be shown in the final poster.

P82 -The Development of a novel tandem fragmentation technique coupled with trapped ion mobility spectrometry and time of flight mass spectrometry for the analysis of protein biomarkers indicative of toxicological threats.

Samuel A. Miller^{*1}, Kevin Jeanne Dit Fouque¹, Benjamin A. Garcia², Francisco Fernandez-Lima¹. ¹Department of Chemistry and Biochemistry, Florida International University, Miami, FL 33199, USA; ²Department of Biochemistry and Molecular Biophysics, Washington University School of Medicine, St. Louis, MO, USA.

Background/Introduction: Toxico-proteomics merges the fields of proteomics and toxicology by the quantification of proteins in biological samples. Proteins are comprised of a series of amino acids which can be altered via functional group addition, otherwise known as post-translational modifications (PTMs). Two primary PTMs are acetylation and methylation, occurring on basic lysine and arginine amino acids of the protein. The PTM additions alter the three-dimensional structure of a protein resulting in activation/deactivation of regulatory or metabolomic mechanisms. Chronic exposure to hazardous heavy metals and chronic alcohol consumption can lead to significant alterations in protein structures, resulting in toxic effects. Changes in protein structure regulate the μ-opioid receptor which subsequently can affect pain sensitivity, dependence, and tolerance of opioids. PTMs occurring in mitochondria also precipitate drug-induced hepatotoxicity. Over 300 unique PTMs have been discovered, and the mapping of toxico-proteomic biomarkers can aid in disease identification, drug compliance and dependence, and other drug-related adverse events.

Objectives: The focus of this research is to develop a novel workflow for the localization of the acetylation and methylation of lysine and arginine residues within protein structures using dual fragmentation methods of ultraviolet photodissociation (UVPD) or collision-induced dissociation (CID) combined with electron capture dissociation (ECD) or a secondary CID, which are coupled to trapped ion mobility spectrometry (TIMS) and time of flight (ToF) mass spectrometry (MS) (UVPD/ CID-TIMS-q-CID/ECD-ToF MS). This workflow is optimized to increase the evaluation of amino acid sequence and localization of PTMs on intact proteins.

Methods: A custom-built Bruker Maxis Impact II q-ToF MS platform (Bruker Daltonics Inc., Billerica, MA) was modified by the addition of a secondary trap-TIMS interface, the installation of an electromagnetostatic (EMS) cell within the collision cell, and the introduction of a 213 nm UV laser (Ekspla, Lithuania). First, the initial intact protein fragmentation efficiency of UVPD vs CID was evaluated. Resulting peptide fragments are then mobility selected via TIMS, quadrupole isolated, and fragmented again by either CID or ECD to generate additional product ions for enhanced amino acid evaluation and PTM localization. Experiments were performed utilizing PTM free intact H4 histone protein standards and H4 histone protein obtained from HeLa cells with induced PTMs. Secondary fragmentation was performed on the isolated c_{30}^{5+} fragment (m/z 621.0) containing the first 30 amino acids in the protein, since the majority of PTMs occur in this section of the histone protein. Data was processed using Data Analysis 5.0 (Bruker Daltonics, Germany) and sequenced using ProSight Lite 1.4 (Northwestern University, Evanston, IL).

Results: Evaluating the efficiency of the initial fragmentation of intact proteins, CID-TIMS-q-ToF MS versus UVPD-TIMSq-ToF MS, the initial fragmentation with UVPD workflow demonstrated 93% sequence identification vs 57% for the CID workflow. Secondary peptide fragmentation compared CID and ECD, with ECD preforming better (~90%) in amino acid sequence evaluation while preserving the PTMs for localization while CID was sufficient (~70%) for sequence evaluation, but can fragment the PTMs such that they cannot be localized. The PTMs in the HeLa cell H4 proteins were successfully identified/localized using the developed UVPD-TIMS-q-ECD-ToF MS/MS workflow on the isolated c_{30}^{5+} peptide, which indicated multiple proteoforms. The developed workflow's combined ability to ion mobility select and quadrupole isolate eliminates possible isomeric/isobaric interferences.

Conclusion/Discussion: Initial UVPD fragmentation coupled with secondary ECD fragmentation provides the greatest amino acid sequence evaluation while retaining PTMs, compared to other dual fragmentation techniques. This analytical workflow effectively and efficiently enabled the tracking of amino acid alterations on proteins that can lead to adverse events.

P83 - Cannabinoid Immunalysis ELISA screening in oral fluid samples using Tecan Freedom Evo 75

Sara B. Jablonski*, MSFS¹, Minjee Kim, MSFS¹, Kahleah Pell¹, Curt E. Harper, Ph.D., F-ABFT¹, Alabama Department of Forensic Sciences, Hoover, AL 35244¹.

Background/Introduction: Tetrahydrocannabinol (THC) and/or metabolites are the most prevalent drugs detected in Driving Under the Influence (DUI) cases in Alabama. Fundamental to forensic toxicology, drug screening is used as a presumptive test which directs confirmation testing. When screening for cannabinoids, several immunoassays use 11-nor-9-carboxy- Δ^9 -tetrahydrocannabinol (COOH-THC) as their assay target. This is not appropriate for oral fluid (OF) screening, as COOH-THC is rarely detected in these samples. Δ^9 -tetrahydrocannabinol (Δ^9 -THC) is the recommended screening target in OF at 4 ng/mL per the National Safety Council (NSC). Using Δ^9 -THC as the target, Immunalysis developed an Enzyme-Linked Immunosorbent Assay (ELISA) specifically for cannabinoid screening in OF. Utilizing this target specific assay allows OF samples with low, biologically relevant Δ^9 -THC concentrations to be identified by immunoassay and subsequently pursued for confirmation testing.

Objectives: To validate the Immunalysis Saliva/Oral Fluid Cannabinoids assay using a semi-automated Tecan Freedom Evo 75 instrument for the use of presumptive cannabinoid screening in OF.

Methods: The laboratory procedure followed was outlined in the Immunalysis kit insert. Immunalysis coated 96-well plates with polyclonal THC antibody. To each well, 25 μ L of OF was added, followed by 25 μ L of pre-incubation buffer. After an hour-long incubation period, 50 μ L of conjugate was added to each well and incubated for 30 minutes to allow for competitive binding. After washing the plate with deionized water to remove matrix and unbound substances, 100 μ L of tetramethylbenzidine was added to the wells and incubated for 20-30 minutes for color development. Hydrochloric acid (100 μ L) was added to stop the color reaction. Optical density was measured at 450 and 620 nm by UV/Vis Spectrophotometry. The cutoff for Δ^9 -THC was set to 4 ng/mL per NSC recommendation. Validation of the assay using the Tecan Freedom Evo 75 instrument included evaluation of parameters per ANSI/ASB 036 Standard Practices for Method Validation in Forensic Toxicology guidelines.

Results: Precision was assessed at four concentrations: 50% below the decision point (2 ng/mL), at the decision point (4 ng/mL), 50% above the decision point (6 ng/mL), and 100% above the decision point (8 ng/mL). Between-day and intra-day precision CVs were less than 10% and 12%, respectively. There were no false positive or false negative results. As a component of the manufacturer's validation, Immunalysis evaluated specificity, cross reactivity, interference, and limit of detection, which were provided in the kit insert.

The Immunalysis OF Cannabinoid assay has been validated and implemented as the cannabinoid screening technique for OF samples at ADFS. Following a negative result, cannabinoid testing is stopped. Confirmation is performed using liquid-liquid extraction with LC/MS/MS analysis. From January 2022 through June 10, 2022, 63 casework samples were analyzed using this screening method. There were no false positive or false negative results. Fifty-two cases screened presumptive positive and were confirmed. Six cases screened negative. Five cases screened negative with elevated responses, and the confirmation results were below the cutoff for the ELISA method. The median Δ^9 -THC concentration was 36 ng/mL, with a range of 1.0 – >300 ng/mL. Cannabinoids detected in confirmation methods include Δ^9 -THC, COOH-THC, OH-THC, cannabidiol (CBD), cannabigerol (CBG), cannabinol (CBN), Δ^8 -THC, and Δ^{10} -THC.

Conclusion/Discussion: Utilizing the Immunalysis Saliva/Oral Fluid assay with Δ^9 -THC target has enabled efficient screening for Δ^9 -THC in OF samples, and added a second technology to our testing workflow. The use of two identification technologies meets proposed standards drafted by OSAC and ASB. To increase cannabinoid detection, a practice may be implemented to assign confirmation testing to OF samples with elevated responses (borderline positives) during screening. In conclusion, laboratories exploring OF testing should select appropriate screening technologies and kits, to ensure adequate detection of cannabinoids in OF.

P84 - Benzodiazepine drug panel screening in hair: development of a screening method at 8 seconds per sample using LDTD-MS/MS

Serge Auger*, Sarah Demers, Jean Lacoursière and Pierre Picard

Phytronix Technologies Inc, Quebec, Canada.

Background/Introduction: During the last few decades, research work has been done to investigate the different drugs of abuse in different types of biological samples (urine, saliva, hair, etc). One of the advantages of hair sample is the noninvasive collection. Laboratories need to screen different drug classes like benzodiazepine, anxiolytic and anticonvulsant using a generic sample preparation and fast analytical technique. The screening of various drug classes requires several different immunoassay reagents or an LC-MS/MS method with a long analysis time. The LDTD-MS/MS technology combines the speed and the analysis of different drug classes within a single method.

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

Methods: Drug-free hair samples from healthy volunteers were used to prepare a screening calibrator. 10 mg hair sample (approximately 1 cm length) were transferred into 2 mL screw cap vials (CK28-R2) having six ceramic beads of 2.8 mm. Hair surface decontamination was performed (1 mL of methanol, soak for 5 minutes, remove methanol then dry). Pulverization of hair sample was done at 6500 rpm (3 X 60 seconds, pause time 15 seconds) using a Precellys 24 system. 1 mL of extraction solvent with internal standard was added and incubated for 1h45 at 60°C for the drug extraction process.

Extracted samples were mixed with phosphate buffer solution, spotted into a LazWell plate and evaporates to dryness before analysis. Drugs were detected in positive MRM mode.

The LDTD-MS/MS system operated in positive MRM mode on a Sciex Q-Trap 5500 mass spectrometer allows for a rapid detection (8 seconds per sample) for 20 different drugs. The drugs are desorbed simultaneously for screening. Specific transitions were monitored for each drug to quantify calibrator levels.

Results: The spiked samples around the decision point and the blank solutions are used to validate the precision of the method. Cut-offs ranging from 125 to 500 pg/mg hair were reached. Each concentration must not exceed 20% CV and the mean concentration ± 2 times the standard deviation must not overlap with other concentrations at the decision point. The peak area against the IS ratio was used to normalize the signal. Replicate extractions are deposited on a LazWell plate and dried before analysis. No overlapping at the decision point is observed for all curves and the %CV were between 1.6 to 12.0% for within-run experiments. For the inter-run, screening calibration curves were evaluated in triplicate over five days. No overlapping at the decision point is observed and the %CV were between 4.2 to 14.1%.

Hair samples were collected from 8 different healthy volunteers and screened to verify the presence of each analyte. Same samples were spiked at two-time cut-off to obtain positive samples. To evaluate the method sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and accuracy, negative and spiked samples were screened with the LDTD-MS/MS method. All negative and positive spiked sample were detected below and higher the screening cut-off, respectively. No false negatives were observed.

Conclusion/Discussion: LDTD combined to Q-Trap 5500 mass spectrometer system allows ultra-fast (8 seconds per sample) screening of drugs in homogenized hair sample using a simple sample preparation.

P85 - SAMHSA drug panel screening in oral fluid: development of a rapid screening method using LDTD-MS/MS and Quantisal[™] device.

Serge Auger*, Sarah Demers, Jean Lacoursière and Pierre Picard

Phytronix Technologies Inc, Quebec, Canada.

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

Objectives: The goal of this presentation is to use an automated sample preparation method for LDTD-MS/MS screening of all compounds in a single operation. Quantisal[™] devices were used for saliva collection.

Methods: Drug-free oral fluids of different volunteers were collected using the Quantisal[™] device. After the collection of the oral fluid, the pad was transferred into a tube containing an extraction buffer. During this process, oral fluids are diluted by a factor of 3. The SAMHSA drug panel for an oral fluid screen was spiked in both extracts at a concentration around the decision point cut-off. Samples were extracted using the Azeo automated extraction system. Due to the potential for ion suppression from the Quantisal[™] extraction buffers, sample preparation was done with salt assisted liquid-liquid extraction (SALLE). Positive ion mode was utilized for the following drugs: amphetamine, methamphetamine, MDA, MDMA, PCP, morphine, hydromorphone, codeine, hydrocodone, cocaine, oxymorphone, oxycodone, 6-MAM and THC.

The following LDTD parameters were used: a laser ramps from 3 seconds to 65% and hold for 2 seconds with a carrier gas (Air) flow rate of 6 L/min. The mass spectrometer operated in MRM mode on Shimadzu LCMS-8060 mass spectrometer. Specific transitions were monitored for each drug to screen samples and standards.

Results: For the method validation, negative saliva samples were spiked at 0.5X, 1X and 2X of the cut-off concentration defined by SAMHSA. Spiked samples and blank solutions were used to validate the precision of the method. The peak area against the internal standard ratio was used to normalize the signal. For all drugs, no overlapping at the cut-off decision point is observed and the %CV was between 3.2% to 15.0% for inter-run experiments. Oral fluids were collected from ten different volunteers. Samples were screened to verify the presence of each analyte (all samples were negatives). Drugs were spiked at 50% cut-off (QC-L) and 200% cut-off (QC-H) and screened as unknown(s) for the cross-validation study.

The LDTD-MS/MS results were used to evaluate the following validation parameters: the method sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and accuracy. A percentage of 100% was obtained for all validation parameters of all drugs.

Conclusion/Discussion: LDTD combined to a Shimadzu LCMS-8060 mass spectrometer system allows ultra-fast (8 seconds per sample) screening of a SAMHSA drug panel in oral fluid using a simple and automated sample preparation method.

P86 - Urine drug monitoring: stress testing a new β -glucuronidase enzyme

Vivek Joshi^{1*}, Mark Powlicki¹, Michael Roberts², Colin Bunner^{2.}

¹EMD Millipore Corp., 400 Summit Drive, Burlington, MA 01803; ²Chem Quant Analytical Solutions, LLC, Apex, NC. *

Background/Introduction: Therapeutic drug monitoring of urine samples is a quick and non-invasive method for monitoring use and abuse of drug classes such as opiates, opioids, and benzodiazepines. Many of these drugs are metabolized and excreted into urine as a glucuronide conjugate form and need to be hydrolyzed before analysis by LC-MS. Enzymatic hydrolysis using β-Glucuronidase followed by LC-MS analysis is common practice in these assays.

Objectives: In this study we evaluate a new pre-buffered, room temperature stable β -Glucuronidase and its efficacy at hydrolysis and recovery of a wide range of analytes over the course of a work week. We also compared the new β -Glucuronidase to a product from an independent vendor. Recoveries for various opioid derivatives using the two different β -Glucuronidase products are reported.

Methods: Drug free urine samples were spiked with different concentrations of mixtures of glucuronide derivatives of opiates/opioids (OP), opioid agonist/antagonists (OPA), benzodiazepines (BENZ), and one antipsychotic (APSY). Master mix was prepared using MS β -Glucuronidase (Sigma: SRE0103), DI water and internal standard solutions in methanol and was stored at room temperature for 120 hours (5 days) while being tested at intervals (0 hours, 24 hours, and 120 hours) to ensure continued efficacy. 180µL of Master mix was combined with 20 µL of urine to initiate hydrolysis reaction. Hydrolysis was carried out at room temp. for 30 min. or 40 °C for 16 min. Post hydrolysis, samples were directly analyzed by LC-MS and recoveries were calculated.

In a separate experiment, Urine samples from different patients (N = 4) were spiked with different concentrations of mixtures of glucuronide derivatives of opiates/opioids (OP) and opioid agonist/antagonists (OPA) and hydrolysis efficacy and recoveries were compared between MS β -Glucuronidase and a commercially available enzyme.

Results: For a range of drugs with different physico-chemical properties as well as chemical classes (Opiates / Opioids, Opioid agonists / antagonists, and Benzodiazepines) greater than 80% recoveries were obtained for all glucuronides within 30 min of hydrolysis at room temp. % CV was typically less than 15% for most of the compounds. Stability study on master mix at room temperature also indicated that the enzyme master mix is stable at room temperature with greater than 80% recoveries throughout 120 Hours (5 days) of storage. This was confirmed by hydrolysis at various time points after storage at room temperature (0 Hour, 24 Hours, and 120 Hours).

When two different glucuronidase enzymes (MS- β -Glucuronidase and β -Glucuronidase from Vendor - 2) were compared for their hydrolysis efficiency for a wide range of glucuronide derivatives from patient urine samples, drug recovery was with in ± 20% for MS – β – Glucuronidase compared to second β – Glucuronidase. This recovery behavior was observed for physico-chemically diverse set of compounds and a wide range of concentrations (20 – 500 ng / ml).

Conclusion/Discussion: Fast and efficient hydrolysis was obtained with high recovery of various analytes and low standard deviation. Consistent recoveries were seen throughout 120-hour storage at room temperature. When compared against a commercially available β – Glucuronidase, similar recoveries were obtained using either enzyme. This implies its capability for multi-day automated sample testing with consistent recoveries and low variability.

P87 - Membrane extraction technology for the analysis of carboxy-THC in urine

Madison Kilpatrick*, William E. Brewer, and Kaylee R. Mastrianni

DPX Technologies, Columbia, SC.

Background/Introduction: In this presentation, we demonstrate the use of the novel C18 membrane extraction technology (MXT) device for extracting delta-9-carboxy-tetrahydrocannabinol (C-THC) from fortified urine samples. A major advantage of MXT is that the device can be utilized with a manual pipettor, a low-cost robotic liquid handler (RLH) with 1 to 8 independent channels, or a high throughput RLH processing 96 samples simultaneously. The device can be coupled to a vacuum manifold to permit seamless sample processing of sample volumes from 50 μ L to 2 or more milliliters, and the process can work with 96 well plates, test tubes, or vials for either GC-MS or LC-MS/MS methods of analysis.

The extraction was performed using β -glucuronidase enzyme for hydrolysis in order to avoid the use of a strong acid (which is known to potentially cause false positive test results with samples containing high levels of cannabidiol metabolites). By using MXT, the enzyme and other sample matrices are removed and not introduced into the LC-MS/MS system, thereby improving the quality of the chromatographic data and minimizing instrument downtime.

Objectives: To learn how to use and develop a new SPE method for analysis of nonpolar drugs and metabolites in urine and other matrices.

Methods:

Sample Preparation

Using a Hamilton Nimbus96 automated liquid handler, a 20µL aliquot of B-One[™] buffer stabilized beta-glucuronidase enzyme solution (Kura Biotech) was added to 50µL urine sample (fortified at various levels of C-THC), 30µL of water, and 10µL of d3-C-THC internal standard solution (0.5 ng/mL in methanol) in a well. The sample was allowed to stand for 10 min at room temperature for hydrolysis.

The C18 MXT device (DPX Technologies) was first conditioned by aspirating and dispensing 100μ L acetonitrile. The solution was added to the membrane and passed through to waste using the pipettor head of the liquid handler. Subsequently, 100μ L of 30% methanol was passed through the MXT device to waste. After this wash step, 100μ L acetonitrile was added to the MXT device and passed through the membrane to elute C-THC (and nonpolar drugs and metabolites). The eluate was diluted with 400 μ L DI water and the well plate was covered.

Analysis

The eluate was injected (5 μ L) into the LC-MS/MS system (SCIEX 6500+) equipped with an Agilent 1260 LC system with a C18 column (Agilent Poroshell, EC-C18, 2.7 μ m, 3x50 mm). The LC method used water with 2mM ammonium formate (A) and 50/50 methanol/acetonitrile (B) for the mobile phases.

Results: The recovery of C-THC in urine was greater than 80% for all concentrations tested, even though the solution pH was not adjusted from the neutral pH hydrolysis conditions. In this method, the calibration plot was linear from 5 ng/mL to 1,000 ng/mL with a linear regression of 0.999. The LOQ was less than 5 ng/mL.

Conclusion/Discussion: MXT with C18 can be used to rapidly and accurately quantitate C-THC in urine with LC-MS/MS analysis. Although many labs are utilizing dilute and shoot methods, maintenance of the instrumentation are often overlooked in terms of the cost per analysis. We are currently developing MXT methods of analysis for other sample matrices such as neat saliva.

P88 - NIST mass spectral reference libraries

William E. Wallace*, Ph.D. Mass Spectrometry Data Center (MSDC), National Institute of Standards and Technology (NIST), Gaithersburg, Maryland.

Abstract: The National Institute of Standards and Technology (formerly the National Bureau of Standards) has been producing mass spectral reference libraries since 1945. Growth accelerated greatly in 1988 when the entire library was digitized and a new computer algorithm was introduced for fully automated library searching. NIST currently produces many types of mass spectral libraries as well as an array of software tools for library searching, data reduction, and quality assurance.

Current libraries include an electron ionization library intended for GC-MS analysis containing spectra of 307,000 organic compounds, an electrospray ionization tandem mass spectral library used for LC-MS analysis containing 31,000 compounds, and several libraries for proteomics which in total contain more than 3 million peptide spectra. Smaller libraries include a DART library of seized drugs, an LC-MS library of glycans (especially those found in human milk), an acylcarnitine library derived from human urine, and a hair and skin keratin peptide library for human identification.

Current software includes the well-known *MS Search* for library searching, *AMDIS* for gas chromatogram deconvolution, *MS Interpreter* for thermochemical ion prediction, *MetaboPique* for liquid chromatogram deconvolution, and Disparate Metabolomics Data Reassembler (*DIMEDR*) which bridges the inconsistencies between multiple LC-MS metabolomics datasets of the same biological sample type taken under different measurement conditions. Additionally, there are a dozen smaller specialty programs all publicly available at the MSDC website <u>chemdata.nist.gov</u>.

The objective of this presentation is to describe to the forensic toxicology community the NIST MSDC offerings and how NIST goes about compiling standard reference data, but, just as important, to learn the needs of the community so that the MSDC can be of better service. Information sought includes classes of compounds not already found in the libraries as well as types of software, or software functions, of particular interest to forensic toxicologists.

P89 - Characterization of hemoglobin covalent adducts by reactive metabolites of cocaine, oxycodone, diazepam, and THC for use as retrospective biomarkers of abused drug exposure

William J. Morrison IV, M.S.*, Anthony P. DeCaprio, Ph.D. Florida International University, International Forensic Science Research Institute, Miami, FL 33199.

Background/Introduction: Hemoglobin (Hb) is a blood protein that contains three unbound cysteine thiol moieties that are nucleophilic and have been shown to covalently adduct to reactive electrophilic xenobiotics. In particular, the β^{93} Cys thiol moiety is highly reactive as it resides on the surface of the protein. While measurement of adducted Hb β^{93} Cys thiols has been employed for environmental and occupational xenobiotics, its use for retrospective exposure assessment for drugs of abuse has not been extensively explored. Furthermore, identification and characterization of covalent Hb thiol adducts is technically difficult, as the level of modified Hb molecules is generally far lower than that of unmodified protein.

Objectives: This study utilized an *in vitro* enzymatic trapping assay combined with a newly developed enrichment method to selectively remove unadducted Hb to allow for a greater sensitivity for identifying and characterizing β^{93} Cys covalent adducts. Additionally, authentic whole blood specimens with confirmed positive identifications were screened to test applicability of Hb β^{93} Cys covalent thiol adducts as a potential biomarker for drugs of abuse.

Methods: The assay utilized human liver microsomes, NADPH, glucose-6-phosphate (G6P), G6P dehydrogenase, and human Hb in ammonium bicarbonate buffer, pH 7.4. Test drugs (cocaine, oxycodone, diazepam, and THC) were added and incubated for 6 h at 37° C. Treated Hb was then subjected to an 18-h incubation with Thiol Sepharose 4B resin for removal of unmodified Hb. Following centrifugation, supernatant containing enriched, drug-modified Hb was collected and an aliquot subjected to tryptic digestion. An Agilent 1290/6530 LC-QTOF-MS in positive mode ESI was used for HRMS peptide analysis. Agilent Qualitative and BioConfirm software, along with Protein Prospector software, were used to process tryptic peptide MS and MS/MS spectral data. Bottom-up proteomic full scan MS data confirmed that covalent modification by reactive drug metabolites occurred at the β^{93} Cys thiol moiety, with mass errors of <5 ppm for adducted species. MS/MS data provided peptide fragments that allowed for structural identification of adducted species. As proof of applicability, authentic whole blood specimens obtained from UTAK Laboratories Inc. were screened using an optimized crash-and-shoot protocol for positive drug identification. Positive specimens were processed for the isolation of Hb protein using HemoVoidTM matrix standard protocol. Isolated Hb was then enriched using a thiol affinity resin and screened on LC-QTOF-MS/MS for Hb covalent adducts.

Results: Tryptic peptide mapping of Full Scan MS data for test drugs cocaine, oxycodone, diazepam, and THC indicated that the specificity of binding site was limited to the Hb β^{93} Cys thiol moiety. No parent drug compounds were found to be the adducted species but reactive metabolites hydroxybenzoylnorecgonine, noroxycodone, hydroxydiazepam, 11-oxo- Δ^{9} -THC, and 9α , 10α -epoxy- Δ^{9} -THC were the covalent adduct species based upon the Δ mass differential found on the tryptic peptide. Follow-up MS/MS data confirmed adduct modification at the β^{93} Cys thiol and provided peptide fragments that allowed for structural identification of covalent modification. Full Scan MS data for the authentic blood specimens revealed that reactive metabolite species were covalently adducted at the β^{93} Cys thiol moiety, with mass errors of <5 ppm.

Conclusion/Discussion: Results of the present study indicate that targeted analysis of covalent Hb modifications as a retrospective biomarker of abused drug exposure could provide an alternative to hair analysis in clinical and forensic toxicological settings. Additional work is being conducted to transfer the method to a LC-QqQ-MS platform for more routine use in forensic laboratories.

P90 - Discovering CYP2D6 activity biomarkers by correlating MDMA pharmacokinetic parameters to untargeted metabolomics data – a proof of concept study

Yannick Wartmann^{1*}, Yasmin Schmid², Matthias E. Liechti², Thomas Kraemer¹, Andrea E. Steuer^{1.1}Department of Forensic Pharmacology and Toxicology, Zurich Institute of Forensic Medicine, University of Zurich, Zurich, Switzerland; ² Division of Clinical Pharmacology and Toxicology, Department of Biomedicine and Department of Clinical Research, University Hospital Basel, Switzerland.

Background/Introduction: Cytochrome P450 (CYP) is a highly relevant enzyme family in forensics. Many members of this enzyme family show huge inter-individual variation in activity not exclusively attributable to the individual's genotype. Commonly, one's exhibited phenotype is determined by the administration of a well-studied CYP substrate and measuring substrate and metabolites in body fluids. However, particularly in postmortem cases this approach is not feasible. Metabolomics techniques, the study of endogenous metabolites, could help to discover biomarkers indicative of an individual's phenotype by comparing the metabolite pattern between different known CYP genotypes/phenotypes. This approach requires a controlled study including both genotyping and probe-substrate phenotyping. Alternatively, pharmacokinetic data of a study in which both a CYP substrate and a CYP inhibitor were administered could be utilized to find correlations between detected features (unidentified metabolites) and the pharmacokinetic parameters of CYP substrate and metabolites.

Objectives: The aim of the current study was to elucidate specific, endogenous metabolites indicative of the metabolic activity of CYP2D6 using an innovative approach combining pharmacokinetics and metabolomics.

Methods: Plasma samples of a controlled MDMA/bupropion cross-over interaction study were used, consisting of four experimental test sessions after a 7-day pretreatment with either placebo or bupropion (placebo-placebo, bupropion–placebo, placebo-MDMA, and bupropion-MDMA, n=16 each). Plasma samples (9 hours) from the test sessions taken from the placebo-placebo and bupropion-placebo groups were protein precipitated and analyzed with a previously published untargeted metabolomics method (Boxler, Drug Test Anal 2019, Vol. 11, Issue 5, Pages 678-696). Briefly, liquid chromatog-raphy followed by a quadrupole time of flight mass analysis (qTOF-MS, Sciex 6600) was performed using a reversed-phase (RP) and HILIC chromatography in both positive and negative electron spray ionization (ESI) mode. Untargeted data was processed using specific software for peak picking and feature identification (msDial 4.8 and SIRIUS 5.5.4). A correlation analysis (spearman) between the area under the curve (AUC) for endogenous features and the previously determined ratio of the AUC of MDMA metabolites divided by the AUC for MDMA was performed. This ratio had been postulated by Steuer *et al.* to predict best the CYP2D6 phenotype (Steuer, PlosOne 2016, Vol. 11, pages e0150955). Filter criteria for unidentified metabolites were fold change between groups of < 0.5 or > 2 and mean signal to noise ratio (S/N) > 10.

Results: In RP positive mode 24858 features and in HILIC negative mode 14286 features were detected. After correlation analysis 123 features (84 RP positive, 39 HILIC negative) with a spearman correlation < -0.55 or > 0.55 and corresponding MS/MS information for identification remained. Three of these features could be identified to be bupropion and its known metabolites, while further 10 features are most likely fragments/metabolites or artefacts of bupropion (typical chlorine cluster). The remaining 110 features could be considered as potential biomarker candidates. Among those, the feature with an m/z of 173.082 was tentatively identified as suberic acid (matched by accurate mass, Dppm of 4.6, and MS/MS information against open-source databases).

Conclusion/Discussion: Using the pharmacokinetic parameters of the CYP2D6 substrate MDMA, with/without CYP2D6 inhibition by bupropion and correlating this data with endogenous metabolites (with/without CYP2D6 inhibition) it could be shown that potential biomarkers for CYP2D6 phenotyping could be discovered confirming our proof of concept. Already, one highly promising marker could be tentatively identified (suberic acid). Of course, it still needs verification in a larger cohort together with other yet unidentified features. Such biomarkers could greatly improve toxicological export reports in assessing concentrations of corresponding drugs. Ideally, a ratio of features that are substrate and product of the enzyme could be established to increase the predictive power of this approach.

P91 - The effect of potential interferences on the Dräger DrugTest 5000

Adrienne Chiang*, Karen L. Woodall. University of Toronto, Mississauga.

Background/Introduction: Oral fluid testing can be a fast, simple and non-invasive way of identifying the presence of drugs for forensic purposes. A positive oral fluid sample may indicate recent drug use and can provide law enforcement with valuable evidence during an impaired driving investigation. In Canada, the Dräger DrugTest 5000 has been approved for use by police officers for the detection of two commonly encountered drugs in impaired driving cases, tetrahydrocannabinol (THC) and cocaine. This device is based on an established scientific test principle known as immunoassay. Immunoassays are subject to cross-reactivity with structurally related or unrelated compounds potentially yielding false positive results in the initial drug screen. Knowledge of these potential interferents is important is determining the reliability of this device.

Objectives: The objective of this research was to evaluate the performance of the Dräger Drug Test 5000[®] by assessing the effects of potential interfering substances (e.g., food and oral hygiene products) on the device performance.

Methods: The potential interfering substances (e.g, mouthwash, food and gum) were chosen based on frequency of use (gum, mouthwash) and media reports of false positive results (poppyseed cake). A total of 30 subjects (n = 19 females, n = 11 males) aged between 18 and 40 years, participated in the study. An oral fluid sample from each individual was obtained prior to the start of testing using a Dräger DrugTest 5000 analyzer and Dräger DrugTest 5000 test cartridge to ensure all subjects that participated in the study did not test positive for cocaine and THC. Once the subject had provided a negative test result, they were assigned to one of three groups (n=10 for each substance). A second oral fluid sample from all three test groups was obtained immediately after exposure to the interferant.

Results: In all test subjects in all test conditions, no positive THC or cocaine results were detected using the Dräger DrugTest 5000.

Potential interfering substance	Test result
Gums	
Trident Splash (Strawberry with Kiwi)	Negative
Trident layers (Strawberry)	Negative
5 (Sour Strawberry Flood)	Negative
Excel (Polar Ice)	Negative
Mouthwash	
Colgate Enamel Health	Negative
Food	
Lemon poppy-seed cake	Negative

Conclusion/Discussion: The results of the study showed that the use of the specific brand of gum, mouthwash and poppyseed cake in this study immediately prior to the test did not produce any false positive results in any test subject for either THC or cocaine. The use of roadside screening for alcohol and/or drugs is routinely challenged in court and the ability of a point of contact screening device to detect the drug in question can be a major issue at trial. The results from this study on the effects of some potential interfering substances on the Dräger DrugTest5000[®] provides useful information about its effectiveness as a reliable screening tool for the investigation of impaired driving.

P92 - Vaping in schools: a comparative analysis of confiscated vaping devices from public-schools central Virginia from 2019 to 2020

Alaina K Holt^{*1,2}, Akira B Goden^{3,4}, Sohee Ha³, Cindy J Miranda³, Alisha Eversole^{3,4}, Caroline O. Cobb^{3,4}, Justin L Poklis⁵, Cosima Hoetger^{3,4,6}, Eric K Soule^{4,7}, Michelle R Peace¹.

¹Department of Forensic Science. ²Integrative Life Sciences Doctoral Program. ³Department of Psychology. ⁴Center for the Study of Tobacco Products. ⁵Department of Pharmacology & Toxicology, Virginia Commonwealth University, Richmond, VA, United States. ⁶Institute for Integrative Health Care and Health Promotion, Faculty of Health/School of Medicine, Witten/Herdecke University, Witten, 58455 Germany. ⁷Department of Health Education and Promotion, East Carolina University, 1000 East 1st Street, Greenville, NC 27858 United States.

Background/Introduction: Use of electronic cigarettes (e-cigs) remains high among youth in the United States, with 11.3% of high school and 2.8% of middle school students reporting past 30-day use in 2021 despite recent federal regulations enacted prohibiting sales to minors under the age of 21. Though underage e-cig use rates reportedly dropped to 13.3% in 2021, down from 18.1% in 2019, e-cig use continues to be a problem in younger populations. Major concerns for underage vaping include risk of lung injury and early dependence to nicotine. Many current e-cig liquids (e-liquids) use nicotine salt formulations. While this allows manufacturers to increase the nicotine content in the e-liquid without increasing the perceived harshness by the user, it also lends to greater nicotine dependence. In addition to known safety concerns from general e-cig use, there is a link between e-cig use and higher prevalence in the use of drugs other than nicotine (DOTNs). Modern e-cig devices have been designed for discreet usage, facilitating the use of e-cigs to vape DOTNs such as cannabidiol (CBD), Δ9-tetrahydrocannabinol (Δ9-THC), heroin, amphetamines, etc.

Objectives: The purpose of this study was to characterize device types and e-liquid composition from products confiscated from middle and high school students in central Virginia public schools to illustrate vaping trends in underage populations.

Methods: Confiscation of products by school resource officers, teachers, and administrators occurred in two public school districts in central Virginia from August 2019 through March 2020. Upon confiscations, a form was filled out documenting confiscation date, student grade level, and any observable physical symptoms from the student whose device was confiscated. Products were submitted to the Virginia Commonwealth University (VCU) Center for the Study of Tobacco Products for initial documentation and coding, then sent to the VCU Laboratory for Forensic Toxicology Research for e-liquid characterization. E-liquids were diluted in methanol for untargeted analysis using Direct Analysis in Real Time Time-of-Flight Mass Spectrometry (DART-MS) and Gas Chromatography Mass Spectrometry (GC-MS), as well as for nicotine quantitation. E-liquids were diluted in water for volatiles analysis using Headspace Gas Chromatography Flame Ionization Detection (HS-GC-FID).

Results: In December 2019, 62 submissions were retrieved from two districts, and in September 2020, 89 submissions were retrieved from one district. The most prevalent e-cig brands and models confiscated were NJOY "Ace" (37.7%); Puff Bar disposable types (17.9%); JUUL (16.6%), Vuse "Alto", "Solo", and "Vibe" (9.3%); Smok "Nord", "Novo", and "Trinity Alpha" (7.3%); and all other brands (11.3%). Most products were confiscated from high school students (53.6%), and e-cig brand was found to differ significantly (p<0.001) by grade status and date of confiscation. Analysis of e-liquids identified nicotine and CBD as pharmacologically active ingredients. Organic acids, a variety of flavorants, degradation products, and other ingredients with unknown purposes were also identified in samples.

Discussion/Conclusions: Findings highlight the popularity of NJOY Ace products, and also a shift away from JUUL products to Puff Bars and other similar disposable devices. The demise of JUUL was possibly due to federal investigations into JUUL's marketing practices, negative media, and US FDA restrictions on flavors in e-liquids in pod-style products. E-liquids were found to range from simple to complex formulations. The presence of organic acids suggests most e-liquids were nicotine salt formulations, which are common in today's market. Reported symptomology, such as "sleepy, pinpoint pupils, slow speech and movement," "shaking," "student threw up," and "under the influence" could not be correlated with the confiscated products based on analytical findings. No other information was collected to indicate or determine if a student had consumed other substances that could also explain symptomology.

P93 - RTI's oral fluid proficiency testing program

Amy Evans^{*1}, E. Dale Hart¹, Dr. Ruth E. Winecker¹. ¹RTI International, Research Triangle Park, NC, 27709, USA.

Background/Introduction: Oral fluid testing has gained recognition in recent years as an alternative matrix for clinical and drug testing laboratories. In addition to the noninvasive collection procedures, oral fluid testing offers detection of ingested drugs, reliable results and limits the ability to adulterate samples. RTI International's Center for Forensic Science (CFS) is accredited by the ANSI National Accreditation board to ISO 17034 and ISO 17043. RTI has been operating an oral fluid proficiency testing program since 2008 to support and advance the quality of oral fluid testing by external assessment of laboratories for quality assurance purposes. Our proficiency testing (PT) program challenges laboratories to a variety of compounds commonly observed in workplace drug testing, clinical and pain management laboratories.

Objectives: Our PT program evaluates quantitative performances of up to twenty-six drug testing laboratories. These laboratories include government and commercial sectors and not all participants are certified by the NLCP for oral fluid testing. Our goal was to observe stability or improvement in the performance of participating laboratories through self-assessment and, where necessary, corrective action.

Methods: PT samples were produced by fortifying a synthetic oral fluid matrix with working drug standards prepared from certified reference materials. Samples were fortified with up to five drug analytes per sample to SOFT 2022 Abstract Submission Form Due by June 10, 2022 challenge participants with an array of drug classes and ethanol. Participants received shipments three times during 2021 and once during 2022. Results were compiled by sample number and calculations were made for the group mean, standard deviation, coefficient of variation (%CV), n, range of results, and the number of results more than 50% from the mean. The performance of each sample and analyte was also evaluated in terms of agreement of the mean to weighed-in (target) value and interlaboratory variability. Participants were provided with summary reports to compare their laboratory performance against a peer group.

Results: Participant mean results agreed well with the target values for most analytes. Of 87 individual analytes across the 20 PT samples, the participant means of 81 (93%) were within \pm 10% of the target value. However, recovery of ethanol was quite low with an average of 76% for the PT samples containing ethanol. Interlaboratory agreement was good with average %CV of 10-15 for 12 analytes (40%) and 15-20 for 16 analytes (53%). Only 2 analytes had an average %CV of greater than 20 – THC (23.6) and Zolpidem (20.4). RTI operated a pilot PT program from 2000-2007 and saw significant improvement in laboratory performance with CV's approaching 5-10% for most analytes. Laboratory performance has remained stable since that time. Improvement can be attributed to a combination of corrective action and discontinuation by the worst performing laboratories.

Conclusion/Discussion: This PT has allowed laboratories to compare their performance against a peer group. For the four PT rounds presented here, the overall performance was very stable with good agreement of group means versus target values for most analytes. Interlaboratory %CV was near 15% for most analytes. The most significant exceptions to our performance measures were the relatively low average recovery of ethanol (76%) versus target and the high average %CV for THC (23.6). While the low recovery of ethanol was unexpected, the high %CV for THC has been a consistent observation over the years, likely caused by homogeneity challenges with THC in an aqueous matrix. Overall, these results demonstrate the benefits for participants wishing to monitor their quantitative performance in comparison to a stable group of laboratories.

P94 - The potential to detect 13 cannabinoid acetate analogs in urine using 6 commercially available cannabinoid homogeneous screening kits

Carl E. Wolf^{1,2}, Alaina K. Holt³, Kimberly N. Karin⁴, Ashley A. Pokhai², Justin L. Poklis⁴, Grace R. Williams¹, Michelle R. Peace². Departments of ¹Pathology, ²Forensic Science, ³Integrative Life Sciences Program, ⁴Pharmacology & Toxicology, Virginia Commonwealth University, Richmond, VA.

Background/Introduction: There has been an exponential surge in the presence and use of cannabinoids since the federal legalization of hemp (Agricultural Improvement Act of 2018), the legalization of medical and adult recreational use and decriminalization of cannabis in several states. This growth has not only been with Δ 9-tetrahydrocannabinol (Δ 9-THC) and cannabidiol (CBD), the most abundant-phytocannabinoid components of cannabis and hemp, respectively, but with many other emerging THC analogs.

Recently, several analogs have become available that are acetate derivatives of various cannabinoids. These analogs are easily synthesized using pyridine and acetic anhydride, the same reagents used to synthesize heroin. They are structurally similar to Δ 9-THC, yet very little information is available about their potency and even less information is available regarding their detectability using the commonly available cannabinoid drug screening assays. Their structural similarity however may be sufficient for the current Δ 9-THC immunoassay screening methods to be able to recognize these cannabinoid analogs.

Objective: To determine the potential of commercially available homogeneous cannabinoid assays to detect the presence of new emerging cannabinoid acetate analogs in urine.

Methods: Acetate analogs were synthesized from available reference materials at a 10 mcg/mL concentration for each analog. Briefly, parent reference standards were dried down, pyridine and acetic anhydride were added, and the standards were incubated overnight in a heat block at 70 °C. Samples were then dried down and reconstituted with methanol. The acetate analogs were Δ9-THC-O, Δ9-HHC-O. Δ9-THCB-O, Δ9-THCP-O, Δ9-THCP-O, exo-Δ9-THC-O, Δ8-THC-O, Δ8-THCP-O, Δ10-THC-O, Δ6a10a-THC-O, CBD-di-O, CBG-di-O, CBN-O. Analog purity was determined by GCMS analysis. Analogs were then prepared individually at 20, 50, 100, and 1000 ng/mL in urine. These analogs were analyzed using 6 commercial homogenous immunoassays, at 50 ng/mL and 20 or 25 ng/mL cutoffs. The federally regulated screening cutoff for Δ9-THC carboxylic acid is 50 ng/mL and in some scenarios (i.e. compliance testing), a lower 20 or 25 ng/mL cutoff is used to further extend the detection window. The immunoassays used were, alphabetically (Tradename (Technique) – Manufacturer): Abbott Cannabinoids (enzyme immunoassay) – Abbott Diagnostics (Abbott), CEDIATM Multi-Level THC (cloned enzyme donor immunoassay) – Thermo Fisher Scientific (CEDIA), DRI^{*} Cannabinoid Assay (enzyme immunoassay) – Thermo Fisher Scientific (DRI), ONLINE DAT Cannabinoid II (kinetic interaction of microparticles in a solution (KIMS)) - Roche Diagnostics (Roche), LZI Cannabinoids (cTHC) Enzyme Immunoassay (enzyme immunoassay) – Lin-Zhi International (LZI), and Syva EMIT^{*} II Plus (enzyme immunoassay) – Siemens Healthineers (Syva). The immunoassays were analyzed on an Abbott Architect c4000 analyzer.

Results: The six homogeneous immunoassays were not able to detect all 13 acetate analogs-and the minimum detectable concentration of the analogs that were detectable was 100 ng/mL. None of the analogs were detected using the immunoassays' 50 ng/mL cutoff concentration.

The Δ 9-THC and Δ 8-THC acetate analogs were detected by the Abbott, DRI and Syva immunoassays using the assays' 20 ng/mL cutoff.

The Δ 9-THCB, exo- Δ 9-THC, Δ 10-THC, Δ 6a10a-THC acetate analogs were detected by the Abbott and DRI immunoassays using the assays' 20 ng/mL cutoff.

Δ9-HHC, Δ9-THCH, Δ9-THCP, Δ8-THCP, CBD, and CBN acetate analogs were not detected by any of the six immunoassays.

Conclusion/Discussion: Some cannabinoid acetate analogs were detectable, but only at the lower cutoff concentration (20 ng/mL), not the federal 50 ng/mL cutoff. Six of the 13 cannabinoid analogs were detectable by the Abbott (20 ng/mL) and DRI (20 ng/mL) assays. Two cannabinoid analogs were detectable by the Syva (20 ng/mL) assay. These analogs were only detectable at or above 100 ng/mL.

P95 - Evaluation of volatile stability in whole blood containing different additives

Ashley Ann Johnson, M.S., MBA^{*}, Haley Melbourn, M.S., Peter Stout, Ph.D., and Dayong Lee, Ph.D. Houston Forensic Science Center, Inc., Houston, TX.

Background/Introduction: Gray top blood collection tubes are preferred for forensic volatiles analysis due to the presence of preservative (i.e., NaF) and anticoagulant (i.e., $K_2C_2O_4$) additives. The supply chain disruptions caused by the COVID-19 pandemic have led to a shortage of various medical devices, including blood collection tubes. Evaluation of different tube types to determine their suitability for analysis of volatiles in blood could provide a suitable alternative if no gray top tubes are available.

Objectives: The purpose of this study is to evaluate the long-term stability of ethanol, methanol, isopropanol, and acetone in whole blood when stored in gray top and 4 alternative color top tubes.

Methods: 10mL gray (100mg NaF, 20mg $K_2C_2O_4$), 4.0mL lavender (7.2mg K_2EDTA), 2.0mL pink (3.6mg K_2EDTA), 2.7mL light blue (0.109M, 3.2% buffered sodium citrate), and 6.0mL clear (no additive) top BD Vacutainer[®] blood collection tubes (Beckton Dickinson, Franklin Lakes, NJ, USA) were filled to approximately 75% (50% for pink) of their fill volume using a syringe with whole blood with citrate phosphate dextrose additive containing 0.08 g/100 mL of ethanol, methanol, isopropanol, and acetone. Volatile stability was evaluated in triplicate for each tube type under two storage conditions (room temperature (RT) and refrigerated) at the following timepoints: 1 day, 2 days, 1 week, 2 weeks, 1 month, and 2 months. The day 1 tubes were also reopened and reanalyzed at each time point. Samples were analyzed using dual column head-space gas chromatography with flame ionization detection with a limit of quantification of 0.01 g/100 mL for all analytes. Acceptable stability is defined by concentrations within the uncertainty of measurement for each analyte (9.6, 11, 9.7, 12% for ethanol, isopropanol, and acetone, respectively).

Results: At 2 months, volatiles from refrigerated tubes, excluding light blue, were within ±4% (unopened) and -9% to 1% (previously opened) of the target concentrations for all except acetone (-12%). Stored at RT, volatiles in unopened gray and lavender tops were within -7% to 4% of the target at 2 months. Under the same conditions, pink tops exhibited acceptable stability at 2 months for methanol and isopropanol (-4% to 3%) and at 2 weeks for ethanol (-9%). However, acetone showed unacceptable stability at 1 month (-19%) but acceptable stability at 2 months (-11%). Volatiles in previously opened day 1 gray, lavender, and pink tops had acceptable stability for 2 months except for ethanol in pink and lavender tops, which exhibited instability after 2 weeks and acetone in pink tops which showed instability after 1 month. Light blue tops showed a decrease by at least 10% for all volatiles after 1 day under both storage conditions. Light blue and clear tops failed to meet qualitative acceptance criteria for ethanol after two days storage at RT. These samples had a peak to valley ratio below 10 for ethanol on the back column due to interference. This interference was not present in refrigerated samples or in the RT samples analyzed on day 1. Volatiles from lavender and pink tops were comparable to gray tops at 2 months.

Conclusion/Discussion: Results indicate that lavender and pink tops have the most comparable stability to gray tops, while light blue tops were the least comparable. Repeated opening of these tubes stored at RT may decrease stability. The immediate decrease in concentration for all volatiles in light blue tops can potentially be attributed to the buffered sodium citrate additive diluting the sample when the tubes were filled. If no other alternatives are available, laboratories should take this finding into consideration when reporting volatiles results in light blue tops as they may be underestimating the volatile concentration.

P96 - Baclofen in postmortem casework (2016-2021)

Brianna L. Peterson*, NMS Labs, Horsham, PA. Lisa Gavin, Clark County Coroner's Office, Las Vegas, NV.

Background/Introduction: Baclofen is a muscle relaxer that is a GABA agonist that reduces the release of excitatory transmitters by binding GABA receptors in the spinal cord. It has been approved for use in the United States since 1977, but most laboratories do not have baclofen, including NMS Labs, as part of their routine scope of testing.

Objectives: This presentation will evaluate the prevalence of baclofen in postmortem forensic toxicology casework performed at NMS Labs from January 2016 through December 2021. Blood concentrations will be reviewed and specific cases will be presented where baclofen was a significant finding in determining the cause of death.

Methods: Baclofen confirmatory analysis was conducted by Liquid Chromatography/Tandem Mass Spectrometry (LC-MS/ MS) with an analytical measurement range of 0.02 - 3.0 mcg/mL in blood. Precursor and product ions utilized were baclofen -214.0 > 151.0 (quantification ion), 214.0 > 144.0 (qualifier); baclofen -D4 - 218.0 > 154.0 (quantification ion), 218.0 > 201.0 (qualifier). Baclofen concentrations were reported to 2 significant figures.

Results: The mean and median concentrations of blood baclofen in the 183 positive cases were 4.1 and 1.0 mcg/mL, respectively. Concentrations (standard deviation) ranged from 0.02 to 41 (7.1) mcg/mL distributed as noted in the Table 1. Published therapeutic blood concentrations for baclofen ranged from 0.10-0.66 mcg/mL, with toxic concentrations ranging from 1.1-3.5 mcg/mL.

Baclofen (mcg/mL)	Ν	%
0.02-0.40	64	35.0
0.41-1.0	29	15.8
1.1-4.0	40	21.9
4.1-10	28	15.3
>10	22	12.0

Т	ā	b	le	1	

There was one outlier of 660 mcg/mL; this case was not included in the statistics. In 15 of the cases, no other drugs were detected. The most common classes of drug detected with baclofen were antidepressants (n=84), anticonvulsants (n=76), opiates (n=75), and benzodiazepines (n=65). The most common co-drug was gabapentin (n=50; drug class = anticonvulsant). Gabapentin and Baclofen are analyzed in the same confirmatory panel; thus 46% of the baclofen/gabapentin co-positives were only determined due to gabapentin being part of routine screening and having the confirmation panel performed. This indicates that baclofen may be more prevalent in casework but is missed due to it not being part of routine screening.

Conclusion/Discussion: The aim of this study was to determine the prevalence of baclofen-associated deaths, alone or in combination with other drugs. This compound is not typically part of routine testing, so there is limited data on typical concentration ranges and co-use of other drugs.

P97 - Prevalence of cannabinoids (delta-8 & delta-9 THC and CBD) in urine from a pain management compliance testing laboratory

Carrol R. Nanco^{*1}, Justin L. Poklis², Grace R. Williams¹, Carl E. Wolf¹. Departments of ¹Pathology, ²Pharmacology & Toxicology, Virginia Commonwealth University, Richmond, VA.

Background/Introduction: Marijuana's main psychoactive constituent, Δ 9-tetrahydrocannabinol (Δ 9-THC), is a federally scheduled drug with medical indications and restrictions dependent on formulation. Many states have legalized marijuana for medicinal and/or adult recreational use. With the passage of the 2018 Farm Bill, commercial products containing other cannabinoids, namely Δ 8-tetrahydrocannabinol (Δ 8-THC) and cannabidiol (CBD), have become readily available. Δ 8-THC is a positional isomer of Δ 9-THC with similar properties. CBD has proposed medical properties. Δ 8-THC is metabolized like Δ 9-THC to the carboxylic acid (Δ 8-THCA) and 11-OH- Δ 8-THC. In the laboratory, immunoassay and confirmatory testing is directed towards the identification of Δ 9-THC/ Δ 9-THCA. The presence of Δ 8-THC, CBD and their metabolites are reported to cross-react with immunoassays, and may also interfere with confirmatory testing. Data has been presented on the presence of Δ 8-THC and CBD in workplace and military drug testing. However, none has been presented on their prevalence in pain management compliance testing.

Objectives: To determine the prevalence of Δ 8-THC, Δ 9-THC, CBD and their metabolites in authentic patient urine samples from a pain management compliance testing laboratory.

Methods: A liquid chromatography–tandem mass spectrometry (UPLC–MS/MS) assay was developed for simultaneous identification of cannabinoids (Δ 9- and Δ 8-THC, Δ 9 and Δ 8-THCA, 11-OH- Δ 9 and Δ 8-THC, CBD and its metabolites, 6-OH CBD, 7-OH CBD, and 7-COOH-CBD.

Calibrators and controls were prepared in drug-free urine; calibrators at 10-1000ng/mL, and controls –low 25ng/mL (parents), 100ng/mL (metabolites), and high -50ng/mL (parents), 500 ng/mL (metabolites). Deuterated internal standards were prepared at 100ng/mL, and 7-OH-CBD-d3 at 50ng/mL in methanol

Samples were prepared by adding 100mcL internal standard to 100mcL urine sample. Cannabinoids were hydrolyzed with β -glucuronidase (*Patella vulgata*) (5000units) incubated at 58°C for 30 min. followed by base hydrolysis (25mcL of 12N NaOH) at 58°C for 30 min. Samples were cooled and neutralized (50mcL glacial acetic acid) and extracted into 250mcL hexane:ethyl acetate (9:1 v/v). Following mixing and centrifugation, the organic solvent was removed and evaporated to dryness. Extracts were reconstituted with 50mcL mobile phase, 5mcL was injected into the UPLC-MS/MS for analysis. The analysis was performed using an AcQuity Xevo TQS-micro UPLC-MS/MS system (Waters Corp. Milford, MA), with a Zorbax (50mmx2.1mmx1.7µm) analytical column (Agilent Technologies, Santa Clara, CA). The mobile phase was: (A) 0.01% ammonium formate in water and (B) 0.01% ammonium formate in methanol. Flow rate was 0.5mL/minute; column temperature was 40°C. Total runtime was 8 minutes.

Authentic urine specimens (n=500) received for pain management testing over a 2-month period were analyzed to determine the prevalence of the cannabinoids. An additional group (n=250) which initially screened positive for Δ 9-THCA at 20 ng/mL cutoff were analyzed for the presence of the cannabinoids, their concentration range, and if present individually or in combination. **Results**: Chromatographic separation was achieved for $\Delta 8$ and $\Delta 9$ -THC, $\Delta 8$ and $\Delta 9$ -THCA, however, 11-hydroxy- $\Delta 9$ and $\Delta 8$ -THC co-eluted. Of the 500 authentic urines, 25% (n=127) were positive for $\Delta 9$ -THC/metabolites; 8% (n=38) were positive for $\Delta 8$ -THC/metabolites, and 6% (n=28) were positive for CBD/metabolites. Of the 250 specimens that screened positive for cannabinoids the following were detected:

Cannabinoid	Δ9-THC	Δ9-THCA	Δ8-THC	∆8-THCA	11-OH-Δ9/Δ8-THC
# of Positives	3	212	1	49	7
Conc. Range (ng/mL)	27-53	12-7,688	25	11-4,012	21-104

Cannabinoid	CBD	7-COOH-CBD	6-OH-CBD	7-OH-CBD
# of Positives	8	11	10	10
Conc. Range (ng/mL)	34-204	20-573	50-494	21-286

Conclusion/Discussion: Δ 8-THC (21%) and CBD (10%) (parent/metabolites) were identified in pain management samples, separately and in combination with Δ 9-THC (parent/metabolites).

P98 - Towards better understanding SCRAs and metabolites in recreational drug intoxications associated with 5F-MDMB-PICA use

Liesl K. Janssens¹, Simon Hudson², David M. Wood^{3,4,} Caitlin Wolfe^{3,5}, Paul I Dargan^{3,4}, **Christophe P. Stove**^{*1, 1} Laboratory of Toxicology, Ghent University, Ghent, Belgium, ² LGC Ltd- Sport and Specialised Analytical Services, Cambridge, UK, ³ Clinical Toxicology, Guy's & St Thomas' NHS Foundation Trust, London, UK, ⁴ Faculty of Life Sciences and Medicine, King's College London, London, UK, ⁵ Department of Emergency Medicine, Dalhousie University, Halifax, NS, Canada.

Background/Introduction: Synthetic cannabinoid receptor agonists (SCRAs) pose a danger to public health. Historically, SCRA use has been associated with a multitude of adverse events, including neurotoxicity and cardiotoxicity. Numerous case reports are published on observed SCRA toxicity, however clear studies on the toxicity of SCRAs in humans are lacking because of obvious ethical reasons.

Objectives: This study focused on a unique cohort of patients experiencing recreational drug toxicity, who had used 5F-MDMB-PICA, to better understand the effects of SCRAs and their metabolites in humans.

Methods: Patient records were evaluated regarding vital signs, Glasgow Coma Scale (GCS) and clinical features. Liquid chromatography coupled to high resolution mass spectrometry (LC-HRMS) confirmed and quantified the presence of 5F-MDMB-PICA (and/or metabolites) as the only SCRA present in the serum of 71 patients.imposing serious health risks. Hence, continuous monitoring of these compounds is important, but is challenged by the rapid emergence of novel analogues that are missed by traditional targeted detection strategies. We addressed this need by performing an activity-based, universal screening on a large set (n = 9681 Cannabinoid activity was assessed in extracts from all serum samples by a cannabinoid receptor (CB1) bioassay, assessing the relationship between serum concentrations and ex vivo human CB1 activation potential. Furthermore, associations with clinical effects were appraised.

Results: 5F-MDMB-PICA and metabolites were pharmacologically profiled in vitro, revealing theoretically possible contributions of two active in vivo metabolites, M2 (oxidative defluorination) and M7 (ester hydrolysis), to overall cannabinoid activity in serum. Serum concentrations of 5F-MDMB-PICA (0.312-69.4 ng/mL) and metabolites (M2=0.081-13.5 ng/mL; M7=0.232-232 ng/mL) were correlated to the ex vivo cannabinoid activity, revealing a sigmoidal relationship. Moreover, the in vitro pharmacological characterization allowed close prediction of an individual's ex vivo CB1 activity. Clinically, the level of consciousness (GCS) showed a significant trend (decrease) with increasing ex vivo cannabinoid activity.

Discussion: Although high concentrations of 5F-MDMB-PICA metabolites (M2 and M7) were present in serum, a negligible contribution of these active metabolites to the overall cannabinoid activity in serum was anticipated, based on their in vitro CB1 receptor activation potential. Prediction of ex vivo cannabinoid activity was made possible by generating a mathematical formula based on the pharmacological characterization of reference standard. Including correction factors within this formula to account for incomplete recovery and matrix effects within the bioassay was necessary. The clinical presentation of 5F-MDMB-PICA-intoxicated patients was not unequivocally correlated to the ex vivo cannabinoid activity that could be measured within their serum. This is in line with previous reports of SCRA misuse resulting in drowsiness or 'zombie-like' effects. The insignificance of other clinical trends, on the other hand, suggests that the clinical presentation pregarding SCRA abuse cannot be deemed as clear-cut. In conclusion, this is the first study to evaluate in a large patient cohort the correlation between possible toxic effects of 5F-MDMB-PICA misuse and ex vivo cannabinoid activity. Pharmacological profiling and in-depth bioassay analysis allowed a better understanding of the contribution of 5F-MDMB-PICA and active metabolites to the total CB1 activity present in serum, suggesting a negligible contribution by metabolites of this specific SCRA despite the high concentrations in serum.

1. Janssens LK, Boeckaerts D, et al. Machine learning to assist in large-scale, activity-based synthetic cannabinoid receptor agonist screening of serum samples. Clin Chem. 2022;hvac027.

P99 - Atypical postmortem redistribution in chronic methadone or fentanyl consumers

Corinne Bouchard*, Béatrice Garneau, Julie Motard, Cynthia Roy, Brigitte Desharnais, Pascal Mireault. Laboratoire de sciences judiciaires et de médecinelégale, Department of Toxicology, 1701 Parthenais St., Montréal, Québec, Canada, H2K 3S7.

Background/Introduction: Postmortem redistribution (PMR) is broadly defined as the alteration of drug concentrations in the body following death. One of the main PMR mechanisms is the diffusion of a substance into blood from surround-ing tissues or organs acting as drug reservoirs. This typically leads to an increase in cardiac or central blood concentration, given its proximity to the liver and gastrointestinal tract. Concentration in the peripheral (e.g., femoral) blood is considered to better mirror the antemortem state, considering its relative isolation. However, lipophilic drugs chronically dosed may subvert those expectations by, e.g., accumulating in the thigh fat and/or muscle which will then act as a reservoir increasing the postmortem femoral blood concentration. Two such instances would be an individual on a methadone maintenance program, used to treat addiction to other opioids, and fentanyl, used as patches to treat chronic pain.

Objectives: This case series presents four postmortem cases where atypical PMR of methadone or fentanyl was observed in chronic users.

Methods: Cardiac and femoral blood were collected and supplemented with sodium fluoride and potassium oxalate for preservation purposes. All samples were stored at 4°C pending analysis. Quantification of methadone and fentanyl was achieved as part of a wide scope confirmation/quantification method, relying on high throughput protein precipitation extraction followed by analysis of the diluted supernatant on an LC-MS/MS (5500 QTRAP[®], Sciex).

Results: In the first two cases, postmortem toxicological analyses were performed on men on methadone maintenance programs. In Case #1, the 57-year-old was hospitalized for liver failure secondary to cirrhosis and died following a sudden decompensation. Analyses revealed methadone concentrations of996 ng/mL in femoral blood and 499 ng/mL in cardiac blood. Antemortem blood, collected at the hospital with heparin additive, was found to contain 418 ng/mL of methadone. In Case #2, a 36-year-old inmate was found to have 1097 and 781 ng/mL of methadone in femoral and cardiac blood, respectively. In the other two cases, 100 µg/h fentanyl patches were used by the victims. In Case #3, a 45-year-old man was found dead in his bed. Fentanyl levels in femoral and cardiac blood were quantified as 48 and 19 ng/mL, respectively. In Case #4, a 67-year-old woman presented to the emergency department and died suddenly after waiting for a few hours. Analyses indicated fentanyl concentrations of 92 and 31 ng/mL in femoral and cardiac blood, respectively. Other toxicological findings were present in all cases, but quantification results did not show the same kind of disparity between central and peripheral blood concentrations.

Conclusion/Discussion: The first case shows a methadone concentration roughly consistent between antemortem and postmortem cardiac blood, but much higher in femoral blood. It has already been noted in the literature that postmortem methadone levels are generally two to four times greater than antemortem levels. More generally though, we find in all these cases a pattern where concentration of a chronically dosed opioid (methadone or fentanyl) is much higher in femoral blood than in cardiac blood. Given the lipophilic nature of these drugs (LogP>2), one hypothesis would be that at steady-state, storage in fat and/or muscle tissue occurs. Following the concentration gradient, this content could be redistributed into femoral blood postmortem. Results from this case series therefore indicate that for chronic users of methadone and fentanyl, great care should be exercised when using femoral blood concentration for interpretation of toxicity. Indeed, depending on the specific case and concentration, a 2-to-3-fold change in concentration might lead to an incorrect conclusion.

P100 - Robotrippin' gone wrong - a dextromethorphan fatality on Miami beach

Diane M. Boland*, Joseph H. Kahl, M. Elizabeth Zaney, George W. Hime, and Katherine L. Kenerson. Miami-Dade Medical Examiner Department, Miami, FL, USA.

Background/Introduction: Dextromethorphan (DXM) is a readily available over-the-counter cough suppressant commonly incorporated into more than 140 cold medications, either alone or in combination with other drugs. The typical antitussive dose for adults is 15 or 30mg, 3 to 4 times per day, with effects persisting for 5 to 6 hours after oral administration. When used as directed, side effects are rarely observed. However, DXM is abused by people of all ages, and is fueled by its over-the-counter availability and accessibility over the internet. When consumed at high doses (over 1500 mg/day), DXM can cause side effects that include sedation, ataxia, hypertonia, miosis and agitation. DXM can also induce a state of psychosis characterized by delusions, hallucinations, and paranoia.

The Toxicology Laboratory at the Miami-Dade Medical Examiner (MDME) reports a case of a 24-year-old black male who, according to law enforcement, was rolling on the ground, screaming, and behaving erratically. Body cam video, however, shows the decedent sometimes calm, controlled, and responsive to questions. After approximately 20 minutes, he was transported to the hospital for treatment, but was pronounced deceased shortly after arrival despite medical intervention. Family members stated the decedent's medical history was unknown; however, he did live a homeless lifestyle and was known to use illicit drugs such as marijuana and use alcoholic beverages. Autopsy findings were relatively non-specific. The decedent had pulmonary congestion, hepatomegaly, hepatic congestion and cutaneous blunt injuries and healing wounds. Brain histology shows subtle findings for Prion Disease; however, this has not been confirmed.

Objectives: The objectives of this presentation are to report a postmortem case involving the ingestion of DXM which includes autopsy and toxicology findings, terminal event behavior, and social history of the decedent.

Methods: Toxicology analysis included a routine screen for volatiles (ethanol, acetone, isopropanol, and methanol) by headspace gas chromatography with flame ionization detection (GC-FID) along with an immunoassay urine screen for amphetamines, benzodiazepines, benzoylecgonine, cannabinoids, opiates, and oxycodone. Additional testing included a comprehensive blood drug screen for over 700 analytes using GC with nitrogen phosphorous detection (NPD) and mass spectrometry (MS) with further confirmation and quantitation by both GC-MS/MS and liquid-chromatography MS/MS.

Results: Toxicology testing revealed the presence of a small amount of ethanol (<0.020%), tetrahydrocannabinol and metabolites, chlorpheniramine, and the following blood and tissue distribution of DXM and its active metabolite, dextrorphan:

SPECIMEN	DEXTROMETHORPHAN	DEXTRORPHAN
Iliac Vein Blood	10 mg/L	0.83 mg/L
Aorta Blood	20 mg/L	1.6 mg/L
Liver	122 mg/kg	3.9 mg/kg
Brain	77 mg/kg	1.7 mg/kg
Gastric	Detected	Detected

Conclusion/Discussion: Therapeutic levels of DXM typically range from 0.001 to 0.6 mg/L in blood in those individuals who are using the drug as directed; however, abusers of DXM who subsequently died due to DXM toxicity resulted in blood levels ranging from 3.3 to 9.2 mg/L and in liver levels ranging from 31 to 230 mg/kg. The case reported by the MDME resulted in a DXM concentration consistent with other reported fatalities. Also noted is the difference in DXM concentration of iliac vein blood vs. aorta blood which supports that DXM may undergo postmortem redistribution. The presence of the active metabolite, dextorphan, likely contributed to the adverse effects of DXM. Based on the autopsy, medicolegal investigation, and toxicology findings, the cause of death was determined to be acute dextromethorphan toxicity with the manner as accident.

P101 - Analysis of drugs in blood to support the UK Section 5A Driving Under the Influence of Drugs Act.

Emily Lee* and Michelle Wood. ¹Toxicology R&D, Waters Corporation, Wilmslow, Cheshire, UK.

Background/Introduction: Many illicit and prescribed medications are reported to impair drivers control of their vehicles, increasing the potential for road traffic accidents. In the UK, since March 2015, introduced legislation changes make it an offence for a driver to have certain drugs, at blood concentrations above specified limits. Analytical testing to support this legislation requires the quantitation of a panel of drugs from differing drug classes, involving a range of chemical properties. To achieve a simple workflow to detect all relevant molecules optimally and at their specified concentrations presents some analytical challenges.

Objective: The aim of this study was to demonstrate efficacy of a simplified sample preparation and UPLC-MS/MS methods developed to meet the requirements of the UK Section 5A of the Road Traffic Act 1988.

Methods: Drug-free whole blood samples were spiked with 17 drug substances listed in the Road Traffic Act, Quality control samples (QCs) at low, medium and high concentrations were also prepared. One hundred microlitres of blood were added to 100µL of zinc sulphate/ammonium acetate, in the wells of an Ostro[™] Pass-Through Sample Preparation Plate (Waters). Samples were eluted with 600µL 0.5% formic acid in acetonitrile containing deuterated internal standards (ISTDs). Two separate aliquots (150µL) of each eluant were taken into a clean 96-well plate collection and dried using an Ultravap Mistral[®] Evaporator. One aliquot for UPLC-MS/MS analysis of THC and one for all other drugs. Two different UP-LC-MS/MS methods were applied in conjunction with a Xevo[™] TQ-S micro mass spectrometer (Waters Corporation) operated in electrospray positive mode; 2 MRM transitions were monitored for each analyte and a single transition monitored for the ISTDs.

The results from the developed method were compared with established, accredited protocols based on two different preparation methods (protein precipitation for basic drugs; liquid/liquid extraction for cannabinoids) and two separate LC-MS/MS methods.

Results: To solve the challenge of acceptable peak shape for polar analytes while maintaining efficient reconstitution for THC, the single pass-through preparation procedure was followed by application of two different chromatography methods. These methods employed the same column (ACQUITYTM BEHTM C_{18}) and mobile phases (0.05% formic acid in water and 0.05% formic acid in acetonitrile) but different chromatographic gradients. Calibrators (seven per analyte plus a blank) typically ranging from 10x below, to 2-5x above, the mandated thresholds, were analysed together with QCs and proficiency samples. Responses were linear (1/x weighting applied) with R² ranging from 0.97 to 0.999. QCs met the applied ±20% criteria and limits of detection met the specified limits for all 17 analytes. Results for proficiency samples showed excellent agreement with the existing procedures.

The developed preparation method was further applied to blood samples spiked with an additional 120 analytes; these included those listed in Section 4 of the UK Road Traffic Act, and other substances that may be relevant for other forensic investigations. Sensitivity data for these are presented.

Conclusion/Discussion: The developed quick and robust method offers a streamlined workflow, replacing two separate preparation methods with one simplified procedure. The method requires just 100μ L blood compared to the current requirement of ~1.5mL. This is beneficial as sample volume can be limited, particularly if multiple analyses are required. The developed sample preparation method has been applied in combination with UPLC-MS/MS for analytes that are covered in Section 4 and 5A of the UK Road Traffic Act and satisfies the legislative requirements. The protocol has also been applied to a wider panel of chemically diverse analytes which may be helpful for other toxicological investigations.

Ostro[™], Xevo[™], ACQUITY[™] and BEH[™] are trademarks of Waters Technologies Corporation. Ultravap Mistral[®] is a registered trademark of Porvair Sciences Limited.

© 2022 Waters Corporation

P102 - An unintentional gamma-hydroxybutyrate (GHB) fatality: be careful of the bottle you drink from

Eric S. Lavins*, Luigino Apollonio, Leslie Dardzinski, Lydia Johnston, Shaena Taylor, Joseph Felo and Thomas Gilson. Cuyahoga County Medical Examiner's Office (CCMEO), Cleveland, OH.

Background/Introduction: Gamma-hydroxybutyrate (GHB) is both an endogenous compound and Schedule I substance. An analog of γ -aminobutyric acid (GABA), it is a cerebral neurotransmitter and is rapidly eliminated from the body, with onset in 10-15 min and a half-life of 30 min. Developed in 1874, it has been used as a general anesthetic, anabolic health adjunct, a recreational drug, and an agent utilized in drug-facilitated crime. GHB and its structural analogs γ -butyrolactone (GBL) and 1,2-butanediol are agonists of GABA_B **receptors** which account for many of the toxicities and are physically and psychologically addictive. GHB is dose dependent usually clear and colorless.

Case: The decedent was a male (20s) staying at a hotel on vacation. A witness informed police that the decedent carried two water bottles, one with water, and the other 1/3 full of GHB. He had reportedly been mixing small doses from the GHB bottle into the regular one, to drink while they were out on the town. That evening, he accidentally poured a supplement packet into the wrong bottle and "chugged it". After vomiting and then appearing normal, he later fell asleep and was found unresponsive. EMS arrived and found the decedent pulseless and in PEA arrest, estimated down for approximately 40 minutes. CPR, epinephrine and NARCAN[®] was administered. On admission he was hypothermic, systolic hypertensive, acidotic with non-reactive pupils. CT scans showed global anoxic brain injury and scattered lung opacities. Acute myocardial injury was suspected secondary to a drug overdose.

Objectives: We describe here an unusual case of an accidental overdose from a street solution of GHB; will discuss the toxicology findings and the history surrounding the case.

Methods: The hospital performed a urine 12-panel immunoassay drug and alcohol screen. Due to prolonged hospitalization, an autopsy was not performed. Toxicology testing was performed on limited admission urine using ELISA, GC/MS, UHPLC-MS/MS and HS-GC-FID. Drug Chemistry performed testing on exhibits utilizing color testing and GC/MS. GHB was quantified in the hospital admission blood by a reference laboratory.

Results: The hospital urine screen was presumptively positive for amphetamines. CCMEO Toxicology testing of the hospital urine confirmed amphetamine, methamphetamine, MDA, MDMA, 7-amino clonazepam, and clonazepam. CCMEO Drug Chemistry identified GHB in the water bottle. NMS Labs confirmed 1400 μ g/mL GHB in the hospital admission blood.

Conclusion/Discussion: It was concluded that the accidental ingestion of GHB was contributory to the intoxication and death. An average street dose of GHB ranges from 1 to 5 grams depending on the purity, making dosing difficult. It has been reported that in lower GHB doses 10 mg/kg bw, euphoria, drowsiness, vertigo, confusion, distress, amnesic effects may be observed. At doses >50 mg/kg bw, the drug can be toxic, resulting in unconsciousness, hypothermia, hypoventilation, vomiting, coma, and death. Published GHB blood concentrations in drivers ranged from 8-600 mg/L, with concentration ranges higher in identified overdose deaths, 30-9200 mg/L.

P103 - Potential detection of 58 fentanyl analogs in urine using fentanyl immunoassays

Modesola A. Akala¹, **Carl E. Wol**f^{1,2}, and Grace R. Williams^{1*}. Department of Pathology¹, Department of Forensic Science², Virginia Commonwealth University, Richmond, VA.

Background/Introduction: The ability to detect fentanyl analogs in urine aids in patient management. From June of 2019 to May of 2020 over 81,000 Americans died from a drug overdose and the United States Drug Enforcement Administration estimates that 75% of those are from fentanyl and fentanyl analogs. Detection enables proper treatment and follow-up potentially including a targeted or untargeted mass spectrometry confirmation assays, naloxone dosage estimation, sociological intervention, and prevalence data for law enforcement. Little is published about the new ARK[™] Fentanyl II Assay formulation's ability to detect fentanyl analogs. Norfentanyl (fentanyl metabolite) cross-reactivity with the ARK II assays is 7%, and the Immunalysis SEFRIA assay is approximately 0.005%. We hypothesize this difference in cross-reactivity extends to the ability to detect different analogs. The purpose of this study was to determine the new ARK II and SEFRIA fentanyl assays' detection of 58 fentanyl analogs.

Methods: 58 purified fentanyl analogs were provided by the Centers for Disease Control and Prevention and divided into four sub-classes: acryl-, butyryl-, furanyl- and thio-fentanyls. The difference in reactivity of both immunoassay's reagents was evaluated in conjunction with the chemical structure of each analog. Each modification is incorporated into one of the four fentanyl molecule moieties; the amide group, aniline ring, piperidine ring, or N-alkyl chain. Some analogs were multiply-substituted. Drug-free urine (DFU) was fortified with 0, 1, 10, or 100ng/mL of the fentanyl analog. Results greater than the 1.0 ng/mL fentanyl calibrator on the Abbott Architect cSystem C8000 were considered positive. Based on reactivity data from the 1 ng/mL concentration, analogs were then tested at 10 or 100ng/mL. Potential trazodone interference was assessed using the primary urine metabolite m-chlorophenylpiperazine (mCPP) at 10, 20, 50, and 100 mcg/mL fortified into DFU. Data Analysis:

Results: Of the 58 analogs tested at ≤100 ng/mL, the ARK II and SEFRIA assays produced 51 and 57 positive results respectively. The cross-reactivity of the assay was predominantly determined by the location of the modification. Modifications to the aniline ring and/or amide group only did not prevent detection by either assay although some did decrease reactivity.

Analogs Detected by Each Assay by Modification Site at ≤100 ng/mL

	Aniline Ring and/or Amide	Piperidine Ring	N-Alkyl Chain
	Group Only (n= 45)	(n= 5)	(n=13)
ARK Fentanyl II	45 (100%)	2 (40%)	5 (38%)
Immunalysis SEFRIA	45 (100%)	4 (80%)	12 (92%)

Of the seven compounds which were undetected by the ARK II assay, all had modifications to the N-alkyl ring and three also had modifications to the piperidine ring. Norsufentanil was not detected by either assay and was the only analog not detected by the SEFRIA assay. DFU fortified with ≤100 mcg/mL mCPP did not result in a false-positive result by either immunoassay.

Conclusion/Discussion: The ARK II and Immunalysis fentanyl immunoassays can detect a range of fentanyl analogs with acryl, butyryl, or furanyl modifications to the amide group or aniline ring of the molecule. N-alkyl chain and piperidine ring modifications significantly affect the ARK II assay's ability to detect the analogs, while the SEFRIA assay appeared less affected and detected all analogs tested except for norsufentanil, which was also not detected by the ARK II assay. In addition to this data set, future work includes testing of an additional 160 fentanyl analogs with both immunoassays. Knowledge of the epitope for each assay may allow for predicted detection of emerging fentanyl analogs. Studies of the false-positive rate of each assay should be tempered by analysis of samples for fentanyl analogs.

P104 - Determination of patient adherence for duloxetine in urine

Haley A Mulder*1, Greg L McIntire², Frank N Wallace², Justin L Poklis³

¹Virginia Commonwealth University, Department of Pharmaceutics, Richmond, VA, ²Ameritox LLC, Greensboro, NC, ³Virginia Commonwealth University, Department of Pharmacology and Toxicology.

Background/Introduction: Duloxetine, known by its brand name, Cymbalta, is a selective serotonin and norepinephrine reuptake inhibitor used to treat major depressive disorders. Duloxetine is regarded as having a good response in the treatment of MDD over its predecessors, tricyclic antidepressants. Despite this, patient adherence for medical treatments in patients diagnosed with MDD and related disorders is a cause for concern. Patients who don't adhere to their dosing recommendations in the treatment of MDD often relapse in their disease state in comparison with patients who adhere to their dosing regimen. Determination of patient compliance for duloxetine is typically determined through medication possession ratio (MPR) or plasma concentrations.

Objectives: The purpose of this research was to characterize normal urinary duloxetine concentrations in patients prescribed duloxetine to monitor patient adherence.

Methods: De-identified patient data collected from routine screens for duloxetine concentrations in urine were included in this study. Samples were diluted and analyzed by an LC-MS/MS validated by the College of American Pathologists (CAP) and Clinical Laboratory Improvements Amendments (CLIA). The use of the results of the de-identified samples for this study was approved as qualified for exemption by the Virginia Commonwealth University Institutional Review Board by the U.S. Code of Federal Regulations (45 CFR 46.102). Inclusion criteria consisted of patients who were prescribed duloxetine and 1) tested positive for duloxetine, 2) tested negative for illicit substances, and 3) included creatinine, age, and duloxetine dose administered. Samples were normalized to creatinine (mg) and dose (mg) and logarithmically transformed. Statistical testing of each parameter was achieved by a pooled t-test of Analysis of Variance (ANOVA). Urinary duloxetine results were broken down by sex, age, body mass index (BMI), and duloxetine dose (mg). Age classifications were broken down as less than (<) 18, 18-64, and greater than (>) 64. BMI ranges were classified as < 18.5 (underweight), 18.5-24.9 (normal weight), 25-29.9 (overweight), and > 30 (obesity).

Results: Of the 5,592 patient urines screened, 2,004 of the results fit into the inclusion criteria. Positive urine concentrations of duloxetine ranged from 50 ng/mL to 2,722 ng/mL. The normalized, transformed urine concentrations of duloxetine ranged from 1.53 to 7.53. Sample distribution included urines collected from 1487 females and 517 males. Age ranges of the specimen donors were between 15 to 90 years old with an average age of 52. BMI levels ranged from 13.9 (underweight) to 88.1 (obese), with the average BMI being 33.5. The most common dose of duloxetine prescribed was a daily, oral dose of 60 mg. Analysis of the normalized, transformed creatinine concentrations showed that there was a significant statistical difference (p < 0.05) in the urinary duloxetine concentrations by sex and by dose (mg). Female patients further showed a statistical difference in urinary duloxetine concentration in age groups 18-64 and 64 and older.

Conclusion/Discussion: By characterizing urinary duloxetine concentrations in patients prescribed the medication, distributions of the normalized data ranges have been established. The normalized, transformed data are significantly different depending on sex, age, an dosing amount. Urine is a clean biological matrix that requires non-invasive collection techniques compared to blood. These data ranges for urinary duloxetine concentrations can be used to determine patient compliance of duloxetine in routine, clinical samples without bloodwork or relying on patient reports.

P105 - Evaluation of multiple oral fluid extractions

Helen Ha*, Samantha Wong, Dani Mata. Orange County Crime Lab, Santa Ana, CA.

Background/Introduction: Many forensic laboratories in the USA are investigating the addition of oral fluid to their analysis scheme for driving under the influence of drugs (DUID) cases. The simple and non-invasive sample collection and ease of handling and storage make oral fluid an ideal specimen for these cases. For laboratories that have not previously tested oral fluids, unfamiliar matrix interferences as well as lower limits of detection (when compared to more traditional blood and urine assays) continue to be large hurdles.

Objectives: To explore multiple extractions and various instrumentation for the qualitative analysisfordelta9-tetrahydroxycannabinoids, methamphetamine, amphetamine, MDMA, MDA, cocaine, benzoylecgonine, cocaethylene, carisoprodol, meprobamate, zolpidem, alprazolam,7-aminoclonazepam,clonazepam, lorazepam, diazepam, nordiazepam, oxazepam, temazepam, codeine, 6-acetylmorphine,buprenorphine, fentanyl, hydrocodone, hydromorphone, methadone, morphine, oxycodone, oxymorphone, and tramadol following the National Safety Council's 2021 recommendations' cutoffs.

Methods:

Instruments

Two instruments were used in this investigation. A Shimadzu Nexera HPLC with an AB Sciex X500 QTOF with mobile phases of methanol and water; both with 0.1% of formic acid. The column consists of Phenomenex Kinetex Phenyl-Hexyl, 2.6 um, 50 x 4.6 mm with UHPLC Phenyl Guard Column for 4.6 mm ID columns, both held at 40 °C for the entire 9.5 minute run time. A Waters XeVo TQ-S with Acquity UPLC(LCMSMS) was also used. The mobile phases were acetonitrile and water, both with 0.1% formic acid. The column used was Phenomenex Kinetex Column, 1.7 um Biphenyl A, 2.1 x 100 mm held at 40 °C for the entire 7.5 minute gradient.

Extraction methods

One in-house protein precipitation method, one published method, dispersive pipette extractions from DPX Technologies and solid phase extraction method, were evaluated. The in-house method started with 0.5 mL of sample and protein precipitated with 1.5 mL cold acetonitrile. Published method of liquid-liquid extraction (LLE) by Krotulski et al. (2018) tested for amphetamines used 0.5 mL of sample, extracted with Borax buffer, N-butyl chloride, ethyl acetate and 10% of hydrochloride acid. Dispersive pipette extraction tested multiple extraction tips: strong cation exchange, weak anion exchange, and a pH balanced at various amounts of sorbent and salt. Extraction methods started with 0.5 mL of sample, extracted with methanol, deionized water, formic acid, 30% of methanol, and tips were eluted with acetonitrile, methanol, and ammonium hydroxide. Solid phase extraction by Marschke (AAFS 2022) used StrataX 33um 60 mg columns and extracted with two washes of water:methanol (95:5) and water:methanol:ammoniumhydroxide (60:39.5:0.5), sample eluted with dichloromethane and isopropanol. All methods included a final dry down and reconstitution in initial mobile phase. Individual steps were modified to optimize the chromatography and resolution for each drug.

Results/Conclusion: All extraction methods were injected on both instruments and Waters LCMSMS in MRM mode detected all the drugs at the concentration recommended due to the ability to adjust parameters for higher injection volume and selectivity for ion fragmentation. Due to the low concentration required for detection, the protein precipitation and LLE method showed poor chromatography for THC. Two extraction methods had acceptable integration and for all drugs resolution: dispersive pipette extraction and solid phase extraction. Solid phase extraction was modified to decrease the reconstitution volume from 0.2 mL to 0.1 mL due to the inability to increase injection volume of the LCMSMS to 20 uL. Dispersive pipette extraction consisted of a simpler procedure comparatively and yielded similar chromatography reading, therefore, additional testing will be with this method.

P106 - A case study on the acute intoxication of strychnine

Helen Ha*Orange County Crime Lab, Santa Ana, CA.

Background/Introduction: Strychnine is an alkaloid found in the seeds from the tree Strychnosnux-vomica located in southern Asia and Australia. High acute exposure to strychnine via the oral and ocular routes can lead to toxicity with symptoms including muscle spasms, hypersensitivity, and respiratory arrest. The infrequent diagnosis of strychnine poisoning in combination with the common adverse effects and rapid onset of effects can result in difficulty treating patients in a timely manner. Strychnine can also be used in drug overdose suicide by purchasing over-the-counter rodent poison. A 32-year-old transient male, height 73 inches, weight 121 pounds, was found outside a bank unresponsive with a large laceration to the top of his head. The autopsy report indicated that the head laceration was superficial, and the respiratory system showed to have moderate to severe pulmonary edema, hemorrhage, and atelectasis.

Objectives: To discuss the common symptoms caused from strychnine poisoning in this case and others in Orange County, CA in the past twenty years. To demonstrate the importance of abroad screening protocol for drugs that are outside of the normal recommended scope.

Methods: In-house toxicology testing confirmed the presence of strychnine and caffeine by a Shimadzu Nexera HPLC with an AB Sciex X500 QTOF. Limit of detection for strychnine was 25 ng/mL, in ESI+ mode. The organic solvent is a mixture of 100% methanol with 0.1% of formic acid, while the aqueous solvent consisted of100% ultra pure water with 0.1% of formic acid. The column used was a Phenomenex Kinetex Phenyl-Hexyl, 2.6 um, 50 x 4.6 mm with UHPLC Phenyl Guard Column for 4.6 mm ID columns, both held at 40 °C for the entire 9.5 minute run time. The LC method started with initial flow of 0.7 mL/min with 90% A and10% B, continue with a linear ramp to switch to 2% A and 98% B till 7.5 minutes, hold till 8.5 minutes. Another linear ramp at the same flow rate switch to 90% A and 10% B to 8.6 minutes, hold till 9.5 minutes. Due to the absence of any other compounds and unknown cause of death, the postmortem blood was sent to a reference laboratory for quantitation of strychnine.

Discussion: Results came back with a concentration of 3.8 mg/L of strychnine in the heart blood. In comparison with referenced articles, concentration of strychnine in fatalities in blood range from 0.5 to 61 mg/L. With the addition of this case, over the last twenty years, our laboratory has seen a total of four cases with strychnine detected. Previous two post mortem cases in our jurisdiction with strychnine in postmortem heart blood had values of 4.6 mg/L and 7.2 mg/L. By transitioning our screening method toa high-resolution mass analyzer in 2017, we gained the ability to back extrapolate our data and to include compounds of interest with ease. This expansion allowed our toxicologists to find uncommon compounds on samples that have unknown cause of death and also to follow current novel psychoactive substances trend in our region.

Conclusion: The cause of death was concluded to be acute strychnine intoxication. The infrequent detection of uncommon drugs can be difficult for death investigation to resolve. The wide spectrum of compounds being screened in our laboratory have assisted in cases where an indeterminate cause of death would have been called previously.

P107 - Evaluation of stop-limit practices for DUI samples with BACs > 0.08 g/100 mL

Jarrod B. Bechard, D-ABFT-FT*, Katelyn N. Harvey, Carrie E. Hodges, D-ABFT-FT. Kansas Bureau of Investigation (KBI) Forensic Science Laboratory, Topeka, Kansas, USA.

Background/Introduction: In the state of Kansas, driving under the influence (DUI) is defined as operating a vehicle with a blood alcohol concentration (BAC) of 0.08 or more, or while under the influence of a drug(s) that renders the person incapable of safely driving a vehicle. There are currently no additional penalties if the individual is driving with a BAC of 0.08 or more <u>and</u> the presence of a drug(s). Preliminary discussions to amend Kansas statute to make this change have begun. For routine DUI blood casework in the KBI Toxicology Laboratory, it is current practice to cancel drug testing if the BAC is greater than 0.08 g/100 mL. Exceptions to this rule include DUI possession cases where evidence is also submitted to the KBI's Drug Chemistry section or when the uncertainty of measurement range falls below 0.08 g/100 mL.

Objectives: Determine the prevalence of driving while under the influence of alcohol in combination with illicit and/or prescription drugs in Kansas drivers. With this information, we will have a better understanding of the potential impact on the laboratory (increased caseload, staffing, supplies, etc.) should current testing practices be modified.

Methods: The agencies with the most toxicology submissions to the KBI were identified and approval was sought to use their case samples for this study. Approved agencies were divided into eight regions to avoid regional drug trends within the state. Samples had to meet the following requirements to qualify for the study: from an approved agency, DUI case (no death investigations, drug-facilitated crimes, etc.), BAC > 0.08 g/100 mL (including full uncertainty of measurement range), drug testing not requested by submitting agency or canceled by laboratory, and the remaining volume of sample greater than 3 mL. Qualified samples were screened via enzyme-linked immunosorbent assay (ELISA) for Barbiturates, Benzodiazepines, Cocaine Metabolites, Methamphetamine/MDMA, Opiates, Oxycodone, PCP, and Cannabinoids.

Results: During the sample collection time frame, 1803 samples were analyzed for DUI blood alcohol testing. Of those 1803 samples, 457 went on for drug testing, 385 had drug testing canceled, and 961 did not request drug testing. Of the 1346 where drug testing was not completed, 435 qualified for the study.

Thirty-eight percent (166) of cases screened positive for at least one class of drugs. Eighteen percent (31) screened positive for more than one class of drugs. The most prevalent drug class was cannabinoids with a 29% (128) positivity rate. An inverse relationship was observed between positivity rates and BAC, with the highest positivity rates occurring when the BAC was less than 0.20 g/100 mL (0.08-0.09, 39%; 0.10-0.19, 40%) and the lowest positivity rates occurring when the BAC was greater than 0.20 g/100 mL (0.20-0.29, 36%; 0.30+, 29%).

Conclusion/Discussion: Nearly 40% of the samples presumptively screened positive for at least one class of drugs. These cases would not have received drug testing with current casework practices. This study was limited to commonly encountered drugs of abuse. Many cases suspected other significant substances that are not covered in this scope, including but not limited to Tramadol, Buprenorphine, Amphetamine, and Mitragynine.

Based on historical submission data, adding drug testing to all DUI cases would increase the laboratory's caseload by 250%, impractical due to budgetary and staffing limitations. However, because of the prevalence of presumptive positives in this study, the laboratory has already modified protocols to add drug testing to cases with a BAC < 0.08 g/100 mL, regardless of request.

P108 - : The quantitation of five antihistamines in blood using LC-MS/MS and the application to human-performance toxicology casework

Kenson Jean*, Kristin W. Kahl, Lisa J. Reidy. Department of Pathology and Laboratory Medicine, University of Miami Miller School of Medicine.

Miami, FL

Background/Introduction: Antihistamines are a class of drugs used to treat allergy and cold symptoms, nausea, and insomnia. Excessive amounts of histamine can cause coughing, congestion, sneezing, and sleeplessness. These drugs function by blocking histamines. First-generation H1 receptor antagonists are a class of antihistamines that can cross the blood-brain barrier and cause symptoms such as drowsiness, dizziness, and blurred vision. These drugs are not scheduled and most are available over the counter. They can have effects that can contribute to impaired driving or drug-facilitated sexual assaults (DFSA). The antihistamines quantitated using this methodology were diphenhydramine, chlorpheniramine, doxylamine, promethazine, and hydroxyzine.

At the University of Miami Toxicology Laboratory, driving under the influence (DUI) cases are first screened for alcohol, if the blood alcohol concentration is < 0.150 g/dL, it will be screened using a sensitive, targeted LC-MS/MS screening methodology and a broad basic drug screen. For DFSA cases, if paired blood and urine are received, the urine will first be analyzed for drugs and alcohol, and then confirmatory tests are performed on both urine and blood. This quantitative blood method complements the sensitive screening methodologies for antihistamines. Eleven cases (4 DUIs and 7 DFSAs) were analyzed using this methodology.

Objectives: The objective was to develop an accurate and reliable quantitative method for five first-generation antihistamines in blood using Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS) and evaluate results for DUI and DFSA cases.

Methods: The method utilizes 500 μ L of blood, fortified with an internal standard solution containing isotopically labeled antihistamines for each compound. The samples are extracted using Cerex[®] **Trace-B solid-phase extraction cartridges. Extracts are analyzed using an Agilent 1260 high-performance liquid chromatography system coupled to an Agilent 6460 triple quadrupole mass spectrometer.** Five μ L of the extracted samples are injected onto the instrument with separation accomplished by gradient elution on an Agilent Poroshell 120 EC-18 (3 x 50mm, 2.7 μ M) column maintained at 45°C. The LC-MS/MS utilizes Jetstream ESI operated in positive, dynamic Multiple Reaction Monitoring (dMRM) mode for identification and quantitation.

Results: The validation of the quantitation of these five antihistamines followed ANSI/ASB Standard 036. All five antihistamines have a limit of detection (LOD) of 0.5 ng/mL and a limit of quantitation (LOQ) of 1 ng/mL. The linear range is 1 to 200 ng/mL with 6 calibration points using a 1/x weighting. The bias for all antihistamines is within ±10% and a %CV of less than 10% for intra- and inter-day precision. Bias and precision were evaluated using low, mid, and high concentration matrix-match controls that were analyzed in triplicate over five runs (n=15). The antihistamines did not demonstrate significant ionization enhancement or suppression.

Antihistamines were quantitated in 11 cases (four DUIs and seven DFSA). Eight out of 11 cases were positive for diphenhydramine with an average concentration of 15 ng/mL and a range of 2.6 ng/mL to 56 ng/mL. Two cases confirmed doxylamine with concentrations of 18 and 26 ng/mL. One case had a hydroxyzine concentration of 2.8 ng/mL. In three DFSA cases analyzed, diphenhydramine was the only drug detected in the blood samples; the other 8 cases reported other drugs in addition to antihistamines.

Conclusion/Discussion: This method achieved low LOD and LOQ concentrations and demonstrated acceptable linearity, bias, precision, and robustness for quantifying five antihistamines in blood.

The detection and quantitation of antihistamines can be significant for the interpretation of these cases, especially diphenhydramine in the DFSA cases. It is important to have an accurate and sensitive method to quantitate these antihistamines at low concentrations in blood due to their availability and effects they may have on human performance. Case interpretations will further be discussed in poster. P109 - Detection of methamphetamine and metabolite in environmental exposure (ChildGuard[®]) cases: a retrospective review.

Liaqat Abbas^{1*}, Kelsey Anderson¹, Donna Coy¹

¹United States Drug Testing Laboratories, Inc, Des Plaines, IL USA.

Background/Introduction: Children living in an environment where drugs of abuse are being used, handled, distributed or manufactured may be exposed at high enough levels that routine drug testing of the child would produce a positive result. United States Drug Testing Laboratories (USDTL) performs environmental exposure testing (ChildGuard[®]) of hair specimens for various governmental agencies as well as family courts around the country. A positive ChildGuard[®] test can suggest, exposure to drugs through smoke, contact with sweat or sebum of a user, contact with drug itself or incidental and/or actual ingestion. Younger children in closer proximity to the user often times test positive for both parent drugs and metabolites.

Objectives: The objective of this study was to investigate positivity trends of ChildGuard samples received at the laboratory based on the age of the donor and the prevalence of parent drugs and metabolites.

Methods: Analysis of ChildGuard[®] specimens received at USDTL between June 1st 2021 thru December 31st 2021 was performed. Results included were positive for methamphetamine and metabolites when the date of birth was available. All specimens were subjected to fully validated immunoassay initial test (ELISA Kit) followed by confirmation. Sample preparation was performed by solid phase extraction, without washing of the hair specimen to preserve detection of environmental exposure. Confirmational analysis by liquid chromatography tandem mass spectrometry (LC-MS/MS) was utilized for all presumptive positives.

Results: A total of 3738 ChildGuard[®] samples were received from June 1st 2021 thru December 31st 2021. In this 7-month period, 2361(63.16%) assays confirmed positive for at least one drug tested. Out of all positives (1694) for amphetamine and methamphetamine testing, date of birth was reported for only 1554 samples. The age group of 0-12 years old was chosen for this study and included 1181 samples. The table below shows the mean, median, and standard deviation of 5 age groups. Concentrations of amphetamine and methamphetamine in hair samples varied across all age groups, with higher concentrations observed in younger age groups.

Age	Median Amp pg/mg	Mean Amp pg/mg	SD Amp pg/mg	Median Methamp pg/mg	Mean Methamp pg/mg	SD Methamp pg/mg
0-0.5 years	181	536.8621	820.355	139	6531.034	9608.615
0.5-2 years	133.5	456.529	973.6097534	2788.5	7681.518	23064.47
2-4 years	67	198.4228188	357.577675	1682	4174.557	6585.182
4-8 years	53	166.5146667	343.7622984	1207	3509.016	7615.885
8-12 years	28	71.5	150.1021	707.5	1478.78	2120.762

Conclusion/Discussion: As our data shows the ratio of amphetamine to methamphetamine decreased as the age increased, which also coincided with an overall decrease in methamphetamine concentrations. This data is consistent with decreased exposure as children become more independent from the caregiver. Hair offers an easy to use tool for testing exposure in children in high-risk situations, is more sensitive, and the detection window is longer than traditional methods like urine or oral fluid. More studies which take more variables of exposure into consideration are needed.

P110 - : Assessing the effect of COVD-19 on the prevalence of ethanol biomarkers in human urine

Lixia Chen*1 and Amanda J. Jenkins 1, 2

¹Quest Diagnostics, Marlborough, MA and ²UMass Memorial Medical Center, Worcester, MA.

Background/Introduction: Ethyl glucuronide [EtG] and ethyl sulfate [EtS] are phase two metabolites of ethanol in humans. Since these compounds have longer half-lives than ethanol, they are useful markers in assessing ethanol exposure. Measurement of EtG and EtS in urine has application in clinical treatment programs such as monitoring abstinence.

Objectives: The objective of this retrospective study was to compare and contrast EtG and EtS concentrations in urine over a period of 3 months in 2 consecutive years, October 1-December 31, 2019, and 2020, from a population in New England, USA. These periods were chosen to assess the effect of the COVID-19 pandemic on prevalence and analyte concentrations.

Methods: Deidentified consecutive random urine specimens for drug monitoring were subjected to immunoassay testing on a Beckman Coulter AU 5800 chemistry analyzer using DRI Ethyl Glucuronide assay [Thermo Scientific] with a cutoff concentration of 500 ng/mL. Presumptive positive samples were evaluated by liquid chromatography-tandem mass spectrometry. Specimens prepared by adding deuterated internal standards using a Hamilton STARlet liquid pippettor were analyzed using a Shimadzu MPX LC20AD/XR with chromatographic resolution by a Phenomenex Luna Omega 5 μ M Polar C18 100 LC Column (50 x 4.6 mm) coupled to a Sciex 4500 mass spectrometer. A multipoint linear calibration curve, with a 1/x² weighting was generated for each run with R>0.990 and included a negative and three positive quality control levels. The clinical reporting range [CRR] for EtG was 500-10,000 ng/mL and for EtS, 100-10,000 ng/mL. EtG was reported only in the presence of EtS.

Results: During the 2019 study period, there were 8673 presumptive positive results. Definitive testing of these resulted in 2505 or 28.9% unconfirmed. Table 1 details the positive findings for EtG and EtS, with approximately 70% of EtS concentrations within the CRR and 54% of EtG concentrations > CRR. For 2020, there were 4274 presumptive positive results, of which 227 or 5.3 % were unconfirmed. Table 2 shows the distribution of positive results, with 68% of EtS concentrations within the CRR and close to 55% EtG concentrations > CRR. In 2019 and 2020, the majority of specimens contained both EtG and EtS [95.6% and 98.6%, respectively].

Table 1. Distribution of EtG/EtS in Positive Urine Specimens with Quantitative Values (ng/mL) in 2019

Drug/metabolite	N, % within CRR	Mean± SEM	Median	N,% >CRR
EtG	2683, 45.9	3389.9±49.6	2499	3159, 54.1
EtS	4325, 70.3	2344.4±38.1	1266	1823, 29.7

Table 2. Distribution of EtG/EtS in Positive Urine Specimens with Quantitative Values (ng/mL) in 2020

Drug/metabolite	N, % within CRR	Mean± SEM	Median	N,% >CRR
EtG	1791, 45.2	3422.5±60.5	2584	2168, 54.8
EtS	2754, 68.1	2384.5±48.3	1281	1293, 31.9

Conclusion/Discussion: The percentage of unconfirmed results was lower in 2020 by 5 fold compared with 2019 [28.9 v. 5.3%]. This may be explained by a parameter change for the immunoassay between study periods with the aim of reducing error [@] flags. The sample volume was decreased and reagent volume increased.

EtG concentrations were greater than EtS and the mean and median concentrations of each were similar for both years, with lower median concentrations compared with the mean for each analyte. For each biomarker, the percentage of positive specimens >clinical reporting range was similar for both years.

The number of confirmed specimens for EtG, EtS decreased by 32% and 34%, respectively from 2019 to 2020. This maybe reflective of the reduction by approximately 27% in the sample volume received in 2020 compared to 2019. There was no change in specimen acceptance criteria during this time frame. In conclusion, although the number of positive specimens decreased in 2020, the pattern of EtG/EtS concentrations was similar for both years.

P111 - Multi-method LCMS assay multiplexing with advanced intelligence capabilities to increase laboratory throughput

Logan Miller*¹, Theresa Meli², Sarah Bartok², Yuka Fujito¹, Kerry Hill¹, Timothy Lee¹, Arthur Harmon-Glaus¹, Andy Sasaki¹, Rachel Lieberman¹. ¹Shimadzu Scientific Instruments, Columbia, MD, ²Quest Diagnostics Nichols Institute, Chantilly, VA.

Background/Introduction: Increased flexibility with analytical instrumentation is an ever-increasing demand within clinical and forensic toxicology laboratories. Multiplex analysis offers significant advantages with respect to time, reagent cost, and the amount of data that can be generated. In high-volume assays, the same analytical method is typically assigned to all streams of a multiplex system to optimize the analysis speed. For middle to low-volume assays, combining multiple methods onto a single LC can maximize the usage of an LCMS system and is highly desirable. A Nexera QX Multiplexing LCMS system, with its advanced hardware and software designs that addresses the common high throughput challenges and simplifies the workflow, was used to analyze psychoactive drugs and synthetic cathinones in human urine.

Objectives: This project demonstrates a workflow that maximizes the functionality of a single LCMS system by conducting multiple assays on a single instrument.

Methods: Two different analytical methods (Method 1: Benzodiazepines in Urine, Method 2: Bath salt in Urine) that require different mobile phases and columns were optimized on an LCMS-8060NX triple quadrupole mass spectrometry system. Both assays were configured and developed to run overlapped on a QX system.

Patient urine samples were extracted following a laboratory established solid-phase extraction procedure for each assay. Chromatography gradients as well as MRM transitions were optimized on both assays for high throughput analysis. Rinsing methods were developed to ensure no carryover in blank samples proceeding the highest-level calibrator for each assay. Injection volumes for each assay were optimized. Reproducibility and accuracy were evaluated and compared before and after the assays were multiplexed.

Results: Autosampler carryover was eliminated by employing a short needle inner rinse program that flushed the needle with a mixture of mobile phase A, mobile phase B, and an organic rinse solvent (40/40/20, ACN/IPA/Acetone with 0.1% formic acid). The rinse program was able to reduce carryover to fall within the acceptance criteria following a high concentration patient sample (greater then 10x ULOQ). Acceptance criteria for carryover, as defined by the laboratory was less than 10% of the cut-off level. The multiple multiplexed assays (2 method, 2 channel overlap) were intuitively run by the system. The result of combining the two assays into one system allows for a reduction in the overall run time of the two batches by over 30 minutes (2 plates of 96 samples sequentially taking approximately 8.6 hours while the overlapping multi method approaching 8 hours). Both assays achieved linear calibration curves with accuracies between 80-120% for each calibration point. Retention times across all the streams were reproducible achieving typically less than 3% RSD. Additionally, because the system can handle multiple column types and mobile phases the system does not require an analyst to change or modify the system's configuration to run multiple methods.

Conclusion/Discussion: Psychoactive drugs and synthetic cathinones in urine were analyzed using a Nexera QX Multiplexing LCMS system. The benzodiazepine and bath salt panel achieved a 2X throughput enhancement by multiplexing the assays together. The QX Solution software interface was used to monitor pressure profiles for each stream and real-time peak area response for each compound/internal standard. This project demonstrates a practical workflow that maximizes the usage a single LCMS system by multiplexing multiple assays without sacrificing data quality while reducing manual intervention.

P112 - Anterograde extrapolation of blood alcohol: comparing Widmark estimations to blood alcohol results obtained in a controlled dosing study

Lucien Lockhart, MS^{1*}; Dana Russell, BS¹; Darby Lanz, MSFS¹; Michael Stypa, MS¹.

¹Las Vegas Metropolitan Police Department, Las Vegas, NV.

Background/Introduction: Anterograde extrapolation is the process of estimating an individual's blood alcohol concentration at a time after drinking (BAC_t). Anterograde extrapolation utilizes the zero-order elimination rate of alcohol in the post absorptive phase and the Widmark equation. A subject is typically post absorptive 1.5 hours after consuming the last drink. Using a controlled dosing study, anterograde extrapolation estimations were compared to actual BAC results.

Objective: This presentation will demonstrate that anterograde extrapolation estimations are reliable when properly utilized.

Methods: A controlled dosing study was conducted involving ten subjects (5 males and 5 females) between the ages of 23-35 years. A breath alcohol test (Intoxilyzer 8000) was administered prior to the start of drinking to verify the absence of alcohol. Distilled liquor was consumed over 1.5-3 hours. Participants were accompanied by a chaperone, who recorded applicable information.

Whole blood specimens were collected from each participant at approximately 3 hours after the end drinking. The blood was collected into 10 mL gray-top tubes and refrigerated prior to analysis. Blood specimens were analyzed using dual column headspace GC-FID (GCHS). The validated method followed ANSI/ASB Standard 036. The uncertainty of measurement (UoM) was 4.6% using a 99.73% coverage probability as determined by the bottom-up approach.

Anterograde extrapolation of BAC was calculated at the time of the blood draw using the information gathered during the study. Values for rho (Male-0.68, Female-0.55 L/kg) and beta (Male-0.015, Female-0.018 g/dL/h) were chosen based on average values published in the literature. Propagation of uncertainty was applied to anterograde calculations with a covariance term (-0.65) because rho and beta are related variables. The results of the propagation of uncertainty were used to compare anterograde calculations to BAC results.

Results: The propagation of uncertainty for anterograde extrapolation included administratively set uncertainty values for the amount of alcohol consumed (A) and time (t). The uncertainty for weight (kg, p) were based on literature references. In males, beta (elimination rate, β) had the most significant uncertainty followed by rho (volume of alcohol distribution, r), and the amount of alcohol consumed. In females, beta and rho shared similar uncertainties, followed by amount of alcohol consumed.

The averaged result of the propagation of uncertainty for anterograde extrapolation of BAC_t was 35% for females and 40% for males.

(2)

Anterograde extrapolation calculations were comparable to BAC results for six of the ten subjects (BAC estimations: 0.10-0.21 g/dL; BAC results: 0.078-0.219 g/dL; percent differences: +/- 4-28%). BAC was overestimated (> 35% in females and 40% in males) for the remaining four participants (BAC estimations: 0.11-0.18 g/dL; BAC results: 0.081-0.127 g/dL; percent differences: +/- 35-100%). These four participants are highlighted in gray in Table 1. Only one of the four was significantly overestimated, most likely the result of a recording error or a higher-than-average elimination rate.

Table 1: Comparison of Anterograde and GCHS Results

	Participant ID	Anterograde Calculations, g /100 mL with Propagation of Uncertainty	GCHS Results, g /100 mL with UoM	Percent Difference
	А	0.17 +/- 0.02	0.151 +/- 0.007	-12.6%
a la	В	0.21 +/- 0.03	0.219 +/- 0.010	4.1%
Val	E	0.18 +/- 0.02	0.090 +/- 0.004	-100.0%
2	F	0.10 +/- 0.02	0.078 +/- 0.004	-28.2%
	Ι	0.14 +/- 0.02	0.120 +/- 0.006	-16.7%
	С	0.15 +/- 0.03	0.111 +/- 0.005	-35.1%
<u>e</u>	D	0.18 +/- 0.03	0.127 +/- 0.006	-41.7%
a la	G	0.17 +/- 0.03	0.137 +/- 0.006	-24.1%
Ъщ	Н	0.17 +/- 0.03	0.167 +/- 0.008	-7.8%
	J	0.11 +/- 0.03	0.081 +/- 0.004	-35.8%

Conclusions/Discussion: Anterograde extrapolation combined with propagation of uncertainty is useful for estimating blood alcohol concentration when sufficient information is available. This science-based approach demonstrates that the largest variations are attributed to beta, rho, and the amount of alcohol consumed. Finally, the result is reported in a range that compares to the low/high estimations of the Widmark equation.
P113 - Death by Chocolate Colored blood – suicidal ingestions of sodium nitrite/nitrate

M. Elizabeth Zaney*, Jacob Samuel, George Hime, Diane M. Boland

Miami-Dade Medical Examiner Department, Miami, Florida.

Background/Introduction: Sodium nitrite (NaNO₂) and sodium nitrate (NaNO₃) are widely available, readily soluble powders that can be purchased online and are commonly used as food preservatives. In "The Peaceful Pill Handbook" sodium nitrite is listed as a quick and simple means to commit suicide. Directions include co-ingestion of an anti-emetic or gastric emptying medication, and suicide kits containing all the necessary ingredients have been described in the literature. Due to the promotion of this methodology in online forums, the suicidal ingestion of these compounds has gained popularity over the past three years in both the United States and other countries. Sodium nitrite and nitrate consumption causes methemoglobinemia by oxidizing hemoglobin to methemoglobin (MetHb) which impairs effective delivery of oxygen to tissues, resulting in hypoxia and death, if untreated. At autopsy the pathologist may observe chocolate colored blood, blue-grey discoloration of the nail beds and a purple/grey/brown skin lividity.

Objectives: To increase awareness of suicide by $NaNO_2$ and $NaNO_3$. To demonstrate what the investigator should look for at the scene, the signs the pathologist may observe at autopsy, toxicology testing that can be performed, and the overall importance of evaluating a case in its entirety.

Methods: Cases selected for testing were based on information gathered from the terminal event scene, information provided by family members, or unusual findings at autopsy (chocolate brown blood and pink lividity). Refrigerated blood samples from tubes containing various preservatives were submitted for MetHb testing on an Instrumentation Laboratory GEM[®]OPL[™]. Confirmatory testing for the presence of nitrite/nitrate was performed on refrigerated ocular fluid samples preserved with sodium fluoride/potassium oxalate (NaF/K₂C₂O₄) using Supelco[®] Nitrate test strips (P/N: 1.10020.0001) and Supelco[®] Nitrite test strips (P/N: 1.10007.001). In-house validation in ocular fluid determined the nitrate limit of detection (LOD) to be 25 mg/L and 5mg/L for nitrite.

Results: The manner of death in all seven cases were ruled as suicides. A summary of the results is shown in the table.

Year & Demographics	MetHb Conc. Blood Source	Test Indic	Strip ation	Other Drugs Detected	Background Informa- tion / Indicators	Cause of Death
2019 49 W F	19% Heart Blood (NaF/K ₂ C ₂ O ₄)	X		Ondansetron, gabapen- tin, mitragynine & 7-OH, yohimbine, duloxetine, 9OH-risperidone, azith- romycin	Information found on decedent's cell phone	Acute Sodium Nitrite Toxicity
2020 18 W M	64% Aorta Blood (NaF/K ₂ C ₂ O ₄)		x	None	Search of decedent's computer	Acute Sodium Nitrate Toxicity
2020 17 W F	79% Antemortem Blood (Sodi- um Citrate)	x		Topiramate, ibuprofen	Suicide note found at scene with mention of sodium nitrite	Acute Sodium Nitrite Intoxication
2021 21 W F	66% Heart Blood (NaF/K ₂ C ₂ O ₄)	x		Quetiapine & nor, ibuprofen, naloxone, terbinafine	Ordered sodium nitrite online	Methemoglobinemia due to Sodium Nitrite Toxicity
2021 48 B F	25% Aorta Blood (EDTA)	x		Meclizine, THC-COOH cocaine metabolites, levamisole, ibuprofen chlorpheniramine	Suicide note, sodium nitrite & OTC meds found at scene	Acute Sodium Nitrite Toxicity
2021 31 W M	17% Aorta Blood (EDTA)		x	Meclizine (8 ng/mL), sertraline & nor, trazodone & mCPP	Search of cell phone and purchases on Amazon	Acute Sodium Nitrite Toxicity
2022 24 B F	17% IVC Blood (EDTA)	x		Metoclopramide	Odd autopsy findings – chocolate brown blood & muscles with a pink hue	Asphyxia due to Methe- moglobinemia due to Ingestion of Unknown Exogenous Oxidizing Agent containing Ni- trite/Nitrate

Conclusion/Discussion: This presentation demonstrates the importance of evaluating all the information when presented with these types of cases. This includes indicators at the scene, additional information from family members, unusual autopsy findings, increased MetHb concentrations, and positive nitrite/nitrate test strip results, to determine an accurate cause and manner of death.

P114 - Analyzing relationships between DRE observations, driving behavior, and THC, methamphetamine, and fentanyl positive toxicology cases in the Michigan Oral Fluid Roadside Analysis Pilot Program

Matthew Levitas^{*1}, Matthew Borr¹, Bridget Lemberg¹, David Lemberg¹, Michael Krause².¹Forensic Fluids Laboratories, Kalamazoo, Michigan;² John Jay College of Criminal Justice, New York, New York.

Background/Introduction: The Michigan Oral Fluid Roadside Analysis Pilot Program was conducted to assess the efficacy and utility of oral fluid roadside testing in Michigan. The program captured valuable data - Drug Recognition Expert (DRE) observations, blood toxicology, and oral fluid toxicology results were obtained for over 500 unique roadside incidents. Forensic Fluids Laboratories analyzed this data-rich information.

Objectives: Our aim was to assess the degree to which a relationship between toxicological results and driving behavior was observable from the data collected using a combination of DRE evaluation and toxicological testing. To assess this relationship, the driving behavior context, observed using the DRE "Reason for Stop", and interaction with DRE opinion were of interest in relation to the likelihood of THC, Methamphetamine, or Fentanyl positive toxicology cases.

Methods: We analyzed the data using a random forest model, Mean Gini decrease statistics, and Bayes Factors to determine if there was an observable relationship between a DRE's reason for stop and incident time, DRE opinion, and toxicological data.

Results: The random forest model yielded 91% accuracy when predicting relationships between reason for stop and the variables we assessed. Bayes Factors represented the likelihood of the DRE reason for stop being impacted by a variable. Reason for stop classified as "failed to yield" and "speed too fast" exhibited Bayes Factors of 7 and 6 with respect to a positive THC oral fluid result. Meaning a positive THC oral fluid result was 7 and 6 times more likely in "failed to yield" and "speed too fast" scenarios. Bayes Factors of 3 and 4.7 are associated in the same way with a positive THC blood result. "Unconscious Driver" exhibited Bayes Factors of 0.93 for positive methamphetamine results in oral fluid and, separately, in blood. "Unconscious Driver" also exhibited Bayes Factors of 0.90 for positive fentanyl oral fluid results, and 1.1 with respect to a positive fentanyl blood result.

Conclusion/Discussion: The variables we analyzed (incident time, DRE opinion, and toxicological data) were accurate predictors of a DRE's reason for stopping a vehicle. The positive THC result in oral fluid and/or blood variables were the most informative in a DRE's reason for stopping a vehicle. When a DRE pulled over a driver based on "failed to yield" or "speed too fast", they were 7 and 6 times more likely respectively to be positive for THC in oral fluid than be negative. A positive THC result in blood was 3 and 4.7 times more likely than being negative. A positive methamphetamine result in oral fluid or blood showed no association with any DRE reasons for stopping a vehicle. When a DRE pulled over a driver for reason of "unconscious driver", a positive fentanyl result in blood was 10% more likely than a negative result. Whereas in oral fluid, there was no increased likelihood of a positive fentanyl result. To the extent which this data set is representative of Michigan drivers at large, these results suggest that speeding and failing to yield are driving behaviors associated with THC use. Overall, oral fluid toxicology results were more informative than blood toxicology results when establishing relationships for a DRE's reason for stopping a vehicle. These likelihoods should be considered and further investigated in future roadside incidents in the state of Michigan.

P115 - Prevalence of designer benzodiazepines in impaired driving casework over a 5-year period (2017-2021)

Michael E. Lamb*, MSFS, D-ABFT-FT; Donna M. Papsun, MS, D-ABFT-FT; Ayako Chan Hosokawa, MS, D-ABFT-FT. NMS Labs, Horsham, Pennsylvania.

Background/Introduction: The detection of designer benzodiazepines (DBZD) in analytical casework has been steadily increasing over the last several years as the class has gained popularity in the recreational drug market. Like other emerging drugs, the identification of these substances is often challenging due to the unique timeline each DBZD persists and lack of routine testing, which is variable across laboratories.

The increase in prevalence of DBZD is of concern to public health and safety, notably cases involving drug impaired driving (DUID). DBZD share anxiolytic, muscle relaxant, and central nervous system (CNS) depressant effects as seen in traditional benzodiazepines. Adverse effects on driving stem from the CNS depressant activity manifesting in increased sedation, confusion, and poor psychomotor skills. Additionally, benzodiazepine use has been demonstrated to enhance effects of other CNS depressants such as ethanol, creating further concern in cases with concomitant drug use.

Objectives: This presentation will show the frequency in detection of DBZD in DUID cases over a five-year time period with supporting casework examples.

Methods: Blood samples sent to NMS Labs for the purpose of DUID investigations and reported positive for at least one DBZD between January 2017 and December 2021, were reviewed. These investigations can be submitted under two tiers of testing, referred to as basic and expanded testing. Basic testing includes a fifteen-panel immunoassay screen. For benzodiazepines, oxazepam is targeted at a cutoff concentration of 20 ng/mL. Cases that screen positive by immunoassay for benzodiazepines are confirmed initially in a panel that involves confirmation for traditional benzodiazepines by LC-MS/ MS (alprazolam, diazepam, clonazepam etc). Expanded panel testing involves the above-mentioned immunoassay screen while additionally screening by LC-TOF/MS using an evolving surveillance library to ensure most relevant substances were identified, including emerging DBZD. Confirmatory analysis for DBZD was performed by LC-MS/MS and quantitated using a standard calibration curve with accompanying controls and/or four-point standard addition.

Results: During the course of this study, at least one DBZD compound was reported in 805 cases submitted by law enforcement agencies for the purpose of DUID investigation. Eleven different DBZD were detected with varying emergence profiles, including three metabolite pairs: etizolam/ alpha-hydroxyetizolam, clonazolam/8-aminoclonazolam, diclaze-pam/delorazepam, flualprazolam, flubromazolam, flubromazepam, bromazolam, and bromazepam. Etizolam/alpha hydroxyetizolam (n=485) and flualprazolam (n=149) were the most frequently detected DBZD followed by clonazolam (n=98) and flubromazolam (n=95).

>90% of cases represented polydrug use with a DBZD present and positive for at least one other compound. Opioids, followed by cannabinoids and stimulants respectively, represented the most popular combinations when a DBZD was reported. Case history and results from DRE assessment (if applicable) were requested when toxicology testing revealed the presence of a DBZD as the only significant finding. Some observations during those assessments included confusions, drowsiness, slow/sluggish reactions, slurred speech and unsteady gait.

Conclusion/Discussion: DBZD has been routinely detected in toxicology casework. Due to the consistent prevalence, it is important to consider the presence of DBZD in DUID casework, especially if confirmations for traditional benzodiazepines are none detected after a positive benzodiazepine immunoassay result. Laboratories must regularly update their scope of analysis to stay current with the changing landscape of drug use, including the increased variety of DBZD being detected in DUID casework.

P116 - Use of the RANDOX biochip array technology as a screening solution to current drug trends in a clinical setting

Pamela Greiss*¹, Laura Keery¹, Victoria Anderson¹, Peter Fitzgerald¹, Lorraine Brigham², Hashem Almahdi², Ji Yeon Kim².¹ Randox Toxicology Ltd, Crumlin, County Antrim, United Kingdom ;² Kaiser Permanente Southern California Regional Reference Laboratories, Los Angeles, CA, USA.

Background/Introduction: Emergency department visit rates because of an opioid overdose increased by 28.5% across the U.S. in 2020, compared to 2018 and 2019¹. The Centers for Disease Control and Prevention (CDC) also recorded **75,673** in the 12-month period ending in April 2021, up from 56,064 the year before and the most opioid overdose deaths ever recorded in the U.S². Overdose deaths from synthetic opioids (primarily fentanyl) and psychostimulants such as meth-amphetamine increased in the 12-month period ending in April 2021. Cocaine deaths also increased, as did deaths from natural and semi-synthetic opioids (such as prescription pain medication)².

Objectives: Randox provides a complete screening solution for these current drug trends in a clinical setting, simultaneously testing for 15 drugs of abuse in urine including Fentanyl, Opioids, Methamphetamine and Cocaine, together with Creatinine as a marker of adulteration by dilution.

Methods: The Randox DOA Urine 16-Plex Array was applied to the fully automated benchtop analyser Evidence MultiSTAT, which processes a self-contained cartridge comprising all the components required for the assays. Results are presented qualitatively by running each sample against a cut off sample of known concentration. Precision and reproducibility of the 15 drug of abuse assays were assessed with a cut off characterisation study. Spiked samples were prepared at -/+50%, -/+75% and -/+100% of the cut off concentration (Cut off; Fentanyl 1ng/ml, Methamphetamine 500ng/ml, Barbiturates 200ng/ml, Oxazepam 200ng/ml, Lorazepam 200ng/ml, Methadone 300ng/ml, Opiates 300ng/ml, BZG/Cocaine 150ng/ ml, Oxycodone 100ng/ml, Tramadol 200ng/ml, THC 50ng/ml, Phencyclidine 25ng/ml, Amphetamine 500ng/ml, Buprenorphine 5ng/ml, and 6-MAM 10ng/ml); each sample was assessed twice a day against the cut off sample, for a total of 20 days (n=40). % Agreement to expected spike value was determined at each concentration.

A total of 611 authentic, unique, and unidentified urine samples collected by Kaiser Permanente Regional Reference Laboratory were assessed on the MultiSTAT DOA Urine 16-Plex Array. All samples had been previously assessed on the LC-MS/ MS confirmatory analysis methods employed by the clinical laboratory. % Agreement to LC-MS/MS was determined for all 15 drug of abuse assays.

Results: For the cut off characterisation study, Fentanyl showed 90% agreement to expected at the -50% concentration and 100% agreement at all other concentrations. For all other analytes, 100% agreement to expected was observed at each of the spiked sample concentrations.

For the authentic sample study, percentage agreement for all 15 assays exceeded 89% demonstrating excellent correlation to LC-MS/MS confirmation methods. Agreements were as follows: Fentanyl 89.7%, Methamphetamine 95.6%, Barbiturates 96.9%, Oxazepam 96.7%, Lorazepam 96.7%, Methadone 99.3%, Opiates 90.4%, BZG/Cocaine 95.3%, Oxycodone 96.9%, Tramadol 99.0%, THC 93.8%, Phencyclidine 94.1%, Amphetamine 94.5%, Buprenorphine 97.9%, and 6-MAM 98.8%.

Conclusion/Discussion: The Randox DOA Urine 16-Plex Biochip Array on the Evidence MultiSTAT analyser demonstrated a high level of precision and reproducibility, together with excellent agreement with current urine LC-MS/MS confirmation methods used in clinical practice. Compared to other pre-configured screening panels, the array has added testing for fentanyl and tramadol, two widely prescribed opioid drugs making it an attractive option for clinical laboratories to meet the needs of their communities during the current opioid crisis³.

References:

- W.E. Soares et al. Annals of Emergency Medicine, 2021; DOI: 10.1016/j.annemergmed.2021.03.013. Emergency Department Visits for Nonfatal Opioid Overdose During the COVID-19 Pandemic Across Six US Health Care Systems.
- 2. https://www.cdc.gov/nchs/pressroom/nchs_press_releases/2021/20211117.htm
- 3. Milone MC. J Med Toxicol. 2012 Dec;8(4):408-16.

P117 - EAGLE FANG: sampling of drug paraphernalia for emerging trends

Olivia Crabtree¹, Hunter Chamma², Kennedy Plude³, Sruthi Ainapurapu⁴, Jordan Casper¹, **Sarah Riley**^{1*.1} St. Louis University School of Medicine, Department of Pathology, St. Louis, MO ; ² St. Louis County Department of Public Health, Office of the Medical Examiner, St. Louis, MO; ³ University of Kentucky, Forensic Toxicology, Lexington, KY; ⁴St. Louis University Forensic Science Undergraduate Program, St. Louis, MO.

Background/Introduction: Postmortem toxicology analysis and interpretation is challenging due to the number of drugs identified in blood or urine from a single individual, including novel psychoactive substances (NPS). It is not possible to determine whether drugs are consumed simultaneously or sequentially from biological matrixes alone. Testing drug material and paraphernalia can provide information on use trends. Evidence And Biological Gathering for Laboratory Evaluation and Forensic Analysis of Novel DruGs (EAGLE FANG) was initiated to identify drugs in material intended for consumption including capsules, tablets, powders, and syringes, or in residue remaining on paraphernalia to determine trends in drug use in the St. Louis, MO metropolitan area.

Objectives: The study was initiated to determine how drugs are being packaged for consumption, comprised of a single drug classes or a mixtures, forms of counterfeit tablets, and trends in drug combinations. The information determined is expected to aid interpretation of postmortem toxicology.

Methods: Physical evidence (products) submitted by the St. Louis County Medical Examiner with postmortem biological specimens were analyzed by gas chromatography-mass spectrometry and liquid chromatography-high resolution mass spectrometry. Plant-like material consistent with marijuana, vitamins, fentanyl or buprenorphine transdermal patches, and cigarettes or similar materials (cigarillos, cigars) were excluded from the study. Each sample was rinsed with methanol, and the methanol rinse loaded on each instrument. For analysis by GC-MS, 2 ml of the methanol rinse was injected on to an Agilent 7890B GC/5977B MSD system. Compound identification after GC-MS analysis was accomplished by spectral comparison to five different libraries, including the Cayman Chemical and SWG-Drug libraries. For analysis by LC-MS, 5 ml was injected on to a Sciex X500R LC-QTOF system. Compound identification was accomplished by Information Dependent Acquisition (IDA).

Results: 271 products submitted between June 2021 and May 2022 met the inclusion criteria. 255 products were analyzed. 33% of the products were identified as prescription drugs, consistent with the product's appearance. 16.1% were a combination of one or more stimulants and one or more opioid. 11.4% were a combination either opioids or stimulants and cutting agents. 6.7% were a combination of stimulants, opioids, and benzodiazepines. Only 6.7% were "pure" substance, i.e., cocaine. 11.4% of products were complex combinations of drugs including opioids, stimulants, antipsychotics, PDE-5 inhibitors, benzodiazepines, and anticonvulsants. The maximum drugs detected in a single specimen was 15, representing 10 different drug classes. Fentanyl analogs detected included acetyl-, acryl-, fluoro-, parachloro-, valeryl-, methoxyacetyl-and butyryl fentanyl. Designer benzodiazepines and hallucinogens were identified. Diphenhydramine, quinine, tramadol, and xylazine were frequently associated with opioids, while lidocaine and levamisole frequently associated with cocaine. Findings correlated with results from biological casework in 92% of cases. NPS were the most often identified in the physical evidence but not on biological material. This could be due to 1) low abundance of these compounds in the physical evidence, 2) insensitivity of the analytical method or 3) the individual did not consume that product.

Conclusion/Discussion: The finding of mixtures including drugs from multiple drug classes in a single product demonstrates that these drugs are used simultaneously. Some cutting agents, like xylazine, tramadol, lidocaine, and levamisole, can have significant toxicity on their own, and forensic experts should be aware of their incidence and distribution patterns. Common illicit drugs and NPS are being mixed with a variety of pharmaceuticals including antipsychotics and anticonvulsants. The identification of NPS in physical material associated with a death scene, but not in likewise associated biological material, has prompted us to evaluate the performance of our screening method. Finally, these findings may be useful for interpretation of postmortem toxicology results.

P118 - Determination of pharmaceuticals and drugs of abuse in the New York/New Jersey Harbor estuary water

Teeshavi Acosta*, Natalie Fernandez, Viviana Chavez, Kate Good, Marta Concheiro. Department of Sciences, John Jay College of Criminal Justice, City University of New York, NY.

Background/Introduction: Pharmaceuticals and drugs of abuse are contaminants of emerging concern, and are increasingly being detected in surface and ground water worldwide. Although consumption of these substances continues to accelerate, there is a lack of knowledge on their impact in the environment and additional research is needed. Many of these drugs were designed to maximize their effect in humans at low levels (ng/mL), therefore their presence in the environment at any concentration pose a significant potential risk to non-target organisms. Additionally, despite degradation in aquatic environments, drugs are considered to be "pseudo-persistent" contaminants due to their continuous use and release into surface waters. Pharmaceuticals and drugs of abuse enter the aquatic environment through a variety of pathways; common routes being through wastewater treatment plant discharge and combined sewer overflows (CSOs). Limited number of studies have investigated the presence of drugs in New York City river water.

Objectives: 1) Develop and validate an analytical method for 28 drugs including commonly prescribed pharmaceuticals (mood altering drugs, cardiovascular drugs, antacid and antibiotic drugs) and high prevalent drugs of abuse (cocaine, amphetamines, opioids and cannabis) in river water; 2) Identify and quantify drugs found in the New York/New Jersey Harbor Estuary Water over the Summer of 2021.

Methods: The authentic river water sample collection was performed through collaboration with local environmental organizations through the New York City Water Trail Association. Samples were collected weekly from 18 locations in the Hudson and East River in New York City from May-Aug 2021 and placed in -20 °C until analysis. Fifty-mL of each river water sample were centrifuged and the acidified supernatant was submitted to mixed mode cation exchange solid phase extraction (SPE), performing 2 separate elutions (basic drugs and THC-COOH). The extracts were analyzed by liquid chromatography tandem mass spectrometry in electrospray in positive mode, utilizing two different gradient separations (basic drugs and THC-COOH) in a C18 reversed phase column. Mobile phase A was 0.1% formic acid in water and mobile phase B 0.1% formic acid in acetonitrile.

Results: The linearity of the method ranged from 5 to 1000 ng/L, and the limit of detection (LOD) was 1 ng/L. The method showed acceptable bias and imprecision. Most analytes demonstrated extraction efficiency values greater than 50%. Regarding matrix effect, most of the analytes displayed ion suppression between -54.8% and -27.8% except for oxycodone, clonidine, 6-acetylmorphine, sulfamethoxazole, cocaine, metoprolol, cocaethylene and methadone that showed no matrix effect.

A total of 215 river water samples were analyzed. The most frequently detected analytes were metoprolol (n=196), benzoylecgonine (n=142), atenolol (n=134) and methamphetamine (n=109), followed by EDDP (n=89), norfentanyl (n=22), methadone, amphetamines, and sulfamethoxazole (n=5 each), cocaethylene (n=2) and fentanyl (n=1). Drug concentrations ranged from LOD to 103 ng/L.

Conclusion/Discussion: We developed a sensitive and specific method for the determination of pharmaceuticals and illicit drugs in river water samples. Common drugs of abuse and prescription medicines were detected in New York City waterways at ng/L levels.

P119 - Suicidal rocuronium toxicity

Teresa R. Gray* and Rafael A. Garcia, Harris County Institute of Forensic Sciences, Houston, Texas.

Background/Introduction: Rocuronium is a neuromuscular blocker used in surgeries to facilitate intubation and provide skeletal muscle relaxation particularly for mechanical ventilation. Rocuronium is a quaternary ammonium compound with limited lipid solubility and minimal oral bioavailability; therefore, intravenous administration is required. Accordingly, onset of action is quick. In clinical settings, patients administered rocuronium are carefully monitored by trained medical staff with access to ventilation and an anticholinesterase. Rocuronium-related fatalities have occurred due to medical error and, infrequently, suicidal intent. Here we report a suicidal administration of rocuronium by a 49 year old woman employed as a surgical nurse. The decedent was found prone in her backyard in a shirt and underwear. Examination of the body at the scene revealed a handgun in the decedent's left hand; however, no firearm injuries were on the body. Medical supplies, including IV start kits, normal saline 0.9% flush injection syringes, and needles were in the decedent's residence. The decedent had a history of migraine, depression, gastroesophageal reflux disease, social alcohol use, and previous suicide attempts by self-administration of oral and intravascular medicines.

Objectives: Describe the pathology findings associated with this fatality as well as an analytical methodology developed to qualitatively identify rocuronium in whole blood.

Methods: A liquid chromatography tandem mass spectrometry (LC-MS/MS) method was developed to qualitatively identify rocuronium. Blood (0.5 mL) was fortified with 20 ng/mL O-desmethylvenlafaxine-D6 then protein precipitated with 1 mL of acetonitrile. Following centrifugation, transferred supernatants dried under nitrogen then reconstituted with the starting mobile phase composition. Extracts were filtered and loaded on an LC-MS/MS. Chromatographic conditions used a C18 column (100 mm X 2 mm X 3.5 μ m) and 7 mM ammonium formate + 0.05% formic acid in water and acetonitrile mobile phases in a gradient. Multiple reaction monitoring mode was used with 530 \rightarrow 488 *m/z* and 530 \rightarrow 70 m/z transitions for rocuronium and 270 \rightarrow 252 and 270 \rightarrow 64 *m/z* transitions for O-desmethylvenlaxine-D6. The method was 3.1 minutes and the limit of detection was 10 ng/mL.

Results: At autopsy, no significant trauma was observed on the body. The external examination was significant for the presence of multiple needle puncture marks overlying subcutaneous veins involving the bilateral upper extremities and the right foot. Internally, slight to moderate changes of hypertensive and atherosclerotic cardiovascular disease, pulmonary congestion and edema (combined lung weight 1,110 g), slight urinary retention (33 mL), and slight to moderate cerebral edema were present. In routine toxicology testing, citalopram (0.22 mg/L), lidocaine, lidocaine metabolite and topiramate were identified; alcohol, opiates, methamphetamine, carisoprodol, cocaine, benzodiazepines, fentanyl, methadone, acetaminophen, other acidic and basic drugs were not detected. Propofol, salicylates and insulin/C-peptide were tested by an external laboratory and found to be none detected or within normal limits. Time of flight mass spectrometry was retrospectively searched and a peak consistent with rocuronium's monoisotopic mass was identified. An in-house method was developed for the identification of rocuronium. Rocuronium was identified in femoral and heart blood well above the limit of detection.

Conclusion/Discussion: The cause of death was attributed to rocuronium toxic effects with subsequent development of respiratory paralysis. The manner of death was classified as suicide. Most previously reported suicides with rocuronium involved medical personnel who presumably had access to rocuronium at their workplace, like the decedent. Postmortem blood concentrations of 1900 and 1500 ng/mL have been reported, consistent with concentrations measured during surgery. In this case, blood was tested qualitatively, as the presence of rocuronium without medical support would be incompatible with survival.

P120 - The incidence of intoxicants in homicide during a surge in homicide deaths

Thomas P. Gilson, MD^{1,2}*, Luigino G. Apollonio PhD^{1,2}, Manreet K. Bhullar, MPH^{1,2}*. ¹Cuyahoga County Medical Examiner's Office, Cleveland, Ohio; ²Cuyahoga County Regional Forensic Science Laboratory, Cleveland, Ohio.

Background/Introduction: Understanding the potential role of intoxicants in violent crime is important to informing public health interventions geared towards decreasing fatalities. In Cuyahoga County, we experienced a significant increase in homicide deaths in 2020 with 252 homicides and 255 in 2021, the highest numbers in the jurisdiction since 1980/1981 and an approximately 40% increase over the years immediately preceding.

Objectives: Identify trends in homicide drug intoxications related to cannabinoids, ethanol, stimulants, opioids and other potential drugs of abuse.

Methods: Homicide cases from the Cuyahoga County Medical Examiner's Office (CCMEO) were examined for a pilot study covering the time period of January to June of 2021.Toxicology reports from the Cuyahoga County Regional Forensic Science Laboratory (CCRFSL) were reviewed for these homicide deaths to survey positive findings of cannabis (as THC and/or metabolites), ethanol, stimulants (including cocaine and/or amphetamine-type substances), opioids, and other potential drugs of abuse (including phencyclidine, benzodiazepines).

Results: In a review of all homicides from January to June of 2021(n = 126), excluding cases under the age of 10, 79.4% of decedents were male, 84.9% were Black, and two thirds (69.0%) of homicides occurred in the city of Cleveland. The average age of decedents was 35.2 years old. 86.7% of all homicides were firearm related homicides. Further examining cases where toxicology testing was completed (n = 117), and excluding cases with no adequate autopsy or hospital samples for testing, we found that 66.7% (78/117) of decedents were positive for cannabis (THC/metabolites), which is only legal for medical purposes in Ohio. Only one of the decedents actually possessed a prescription for medical marijuana. Ethanol, a legal intoxicant, was confirmed in 47.9% (56/117) of decedents, and 34.2% (40/117) of decedents were positive for stimulants (cocaine and/or amphetamine-type substances). Opioids were present in 17.1% (20/117) of cases, and 13.7% (16/117) of cases had other drugs present (benzodiazepines, hallucinogens). Over half (53.0%) of decedents had an average of two or more intoxicants detected. 27.4% (32/117) decedents had both ethanol and cannabinoids present, and 17.1% of decedents had ethanol and stimulants present.

Conclusion/Discussion: More than half had postmortem toxicology testing positive for cannabinoids, while just under half had ethanol detected and one third tested positive for stimulants. There was a notable overlap in these numbers, as decedents who had any intoxicants present had an average of two. While the role of cannabinoids in violent crime is open to debate, the identification of cannabis as the most prevalent intoxicant in area homicides merits further discussion. The overlap across use of cannabis, ethanol, and particularly, illicit stimulant use, is of additional interest in terms of behavioral effect(s), impacts in the community, and points for intervention. This presentation will further explore the public health implications of these intoxicants in homicide victims.

DON'T FORGET TO CLAIM YOUR CONTINUING EDUCATION CREDIT FOR SOFT 2022!

Continuing Education certificates are povided by The American Association for Clinical Chemistry (AACC). You will be emailed a link within two weeks of SOFT 2022 so you can claim your CE certificate. Upon following the link, you will be prompted to log into your AACC account OR create a free AACC account if you do not already have one. Please follow the prompts to evaluate the workshop and/or scientific sessions and confirm your demographic information. Once completed you will be provided with a printable/savable PDF certificate and an electronic copy will be saved to your AACC account. If you have questions or need assistance with your AACC account, please contact them directly here:

AACC

900 Seventh Street, NW Suite 400 Washington, DC 20001 Main Phone: 1.800.892.1400 or 1.202.857.0717 Customer Services Department: 1.800.892.1400 or 1.202.857.0717, option 2

NOTES

NOTES

NOTES

Thank you to our 2022 Exhibitors!

We greatly appreciate your support of SOFT and our toxicology community. Be sure to stop into the hall and say hi to

your favorite exhibitor and play the exhibitor trivia game.

*First Time Exhibitor

Be sure to stop by their booth and give them a warm SOFT welcome!

322	Abbott			
422	Agilent Technologies			
312	Alternative Biomedical Solutions			
730	American Molecular Laboratories*			
411	American Solutions for Business			
628	ANSI National Accreditation Board (ANAB)			
316	ARK Diagnostics			
614	Axiom Diagnostics			
727	Biochemical Diagnostics/KOVA Intl			
721	Biotage			
427	Bruker Scientific			
715	Campbell Science			
625	Cayman Chemical Company			
307	Center for Forensic Science Research and Education			
728	ChromTech			
405	Clinisys*			
724	College of American Pathologists			
311	Data Unlimited International			
328	DPX Technologies			
522	FINDEN Kura			
414	Forensic Advantage			
529	Full Spectrum			
314	GenTech Scientific			
412	Golden West Diagnostics			
527	Grenova			
428	IMCS			
423	Indigo BioAutomation			
324	INTEGRA Biosciences			
425	ionBench*			
323	JEOL USA			
327	JG Finneran*			
526	JusticeTrax			
720	Lipomed			
413	MilliporeSigma			
622	National Forensic Laboratory Information System			
505	Neogen			
406	NMS Labs			
632	Omega Laboratories*			
524	OraSure Technologies			
624	Peak Scientific			

325	PerkinElmer
305	Phenomenex
404	Phytronix Technologies
514	Randox Toxicology
306	Regis Technologies
416	Restek
504	SCIEX
308	Sciteck Diagnostics
714	Shimadzu Scientific Instruments
310	Siemens Healthineers
630	SteelFusion
507	Tecan
530	Thermo Fisher Scientific
511	UCT
321	UTAK
623	Validity Diagnostics
313	Waters

SOFT 2022 Exhibit Hall Hours Exhibit Hall A Tuesday, November 1, 6:30-9:30 pm Wednesday, November 2, 9:30 am-4 pm Thursday, November 3, 9:30 am-3:30 pm

TIER 1 TIER 2 TIER 3 TIER 4 TIER 5



TIER 1

Abbott Agilent Technologies Randox Toxicology SCIEX Shimadzu Scientific Instruments Thermo Fisher Scientific UCT

TIER 2

Axiom Diagnostics Bruker Scientific Campbell Science FINDEN Kura MilliporeSigma Restek UTAK Waters

TIER 3

Clinisys Golden West Diagnostics Lipomed

TIER 4

ARK Diagnostics Biotage Indigo BioAutomation Tecan

TIER 5

JEOL USA Neogen Peak Scientific

EXHIBITORS

Abbott Erika Frank, Sr. Product Manager <u>Erika.frank@abbott.com</u>

https://immunalysis.com/

There is nothing more personal than our health. With a comprehensive range of toxicology reagents, Abbott's brand of Immunalysis™ assays equips you with relevant tools to support the health and well-being of your patients.

Abbott Immunalysis develops and manufactures immunoassay reagents for the detection of drugs and alcohol in a variety of biological matrices such as oral fluid, urine, and blood for use in clinical, criminal justice, workplace, and drug treatment settings.

Our reagents are provided in three ready-to-use formats (our exclusive HEIA[™], high-sensitivity SEFRIA[™], and classic ELISA) and are available in multiple kit sizes including convenient 60 mL wedges for use on high-volume analyzers. With the largest panel of ELISA and HEIA oral fluid reagents, Abbott also offers Quantisal[™], the leading oral fluid collection device with an easy-to-read volume adequacy indicator.

Our complete line of assays, calibrators, and controls provides cost-effective and efficient drug testing solutions for today's laboratories.

American Solutions for Business

Contact Person: Kathy Petrick / KathyPetrick@msn.com 901 Penamint Court Chanhassen, MN 55317 Direct Phone: 952-470-1020

American Solutions for Business is a leading distributor of custom business documents and labels including **Federal and Non-Federal Chain of Custody forms** and labels for Forensic Toxicology, Pain Management, and many other laboratory applications and markets. We provide accession labels, tamper evident labels and other solutions designed specifically for laboratories including freezer grade adhesives. Our comprehensive laboratory program offers high quality form and label products, including a universal forensic all-in-one label pod, leading edge technologies including OCR scanable documents, and labels that provide a solid and defendable adhesive performance for laboratories.

We provide an exceptional level of customer service, an attractive pricing structure, and Program Manager – Kathy Petrick, who has been dedicated to the toxicology and drugs of abuse industry for over 25 years.

American proudly provides a variety of promotional products including backpacks, bags, drinkware, socks, lunch totes, lapel pins, writing instruments, custom decorated apparel and so much more. American has been selected by SOFT for the past several years to provide the SOFT Conference with promotional products and conference apparel.

Come get the "411" about forms, labels and promotional products and see Kathy Petrick at booth 411 to learn more.

THE ANSWERS ARE OUT THERE. LET US HELP YOU FIND THEM!

Axiom Diagnostics, Inc.

www.axiomdiagnostics.com 4309 West Tyson Avenue Tampa, FL 33611 Phone: 888-837-8783 or 813-902-9888

Contact Person: Jesse Carter

Axiom Diagnostics's Test True[™] products detect and identify adulterants in urine samples submitted for drugs of abuse urine (DAU) testing at unbeatable prices. For excellent quality try Test True[™] assays for nitrite, pH, specific gravity, chro-

mate, creatinine, aldehydes, oxidants, and our newest test kit for synthetic urine. Our synthetic urine assay effectively identifies 95% of all synthetic urine products currently on the market. No other synthetic urine marker is as effective as Axiom Diagnostics Test True[™] Synthetic Urine Assay (see MRO Alert, Oct. 2015, Vol. 26, No. 8, p2). Axiom's Test True[™] Oxidant Assay identifies many oxidants including nitrite, chromate, bleach, iodic acid, iodate, and peroxide. Published research has shown that Axiom's Oxidant Assay has out-performed the competition hands down (see MRO Alert, Oct. 2013, Vol. 24, No. 8, p4). For reprints of these articles visit our booth, call our office or see our website. The TrueTrol multi-level controls continually evolve to meet SAMHSA QA requirements. Our specific gravity controls meet SAMHSA QA requirements for refractometer as well as our own SG assay. Don't see what you need? Custom made controls and product packaging are no problem. Fast and friendly service is as easy as dialing toll free 888-TESTRUE (888-837-8783) where you will always get a helpful human on the line ... no electronic call screening ever!

Stop by our booth to learn more about our latest innovations, including our Synthetic Urine Detection Assay. Don't settle for second best! Axiom's Test True[™] products provide the BEST results. Don't miss our annual wine give away. Stop by and say hello and register to win a surprise gift!

Bruker Mass Spectrometry Richard Seitz richard.t.seitz@bruker.com www.bruker.com

Bruker Mass Spectrometry Solutions for Forensic Toxicology and Drug Identification

Introducing TargetScreener 4D – the 4th analytical dimension is going mainstream

TargetScreener is Bruker's answer to the growing need for an integrated HRMS solution for complex toxicology and drug identification tasks right out of the box. Combining state-of-the-art instrumentation, a highly curated database, and powerful, easy to use software for both targeted and non-targeted workflows, TargetScreener provides capabilities for simultaneous detection and quantitation of multiple targets in different matrices. Data acquisition methodology also supports retrospective analyses and non-targeted discovery screening to deliver critical answers today and prepare for unexpected challenges tomorrow.

TargetScreener 4D is designed for use with Bruker's cutting edge timsTOF MS instruments. Trapped Ion Mobility Spectrometry (TIMS) allows for faster analysis, increased sensitivity, and improved specificity by enabling the additional measurement of Collisional Cross Section (CCS). CCS values are the fourth identification criteria integrated into Target-Screener 4D along with retention time, mass spectrum, and intensity. This additional layer of confidence in identification provides better structural confirmations, better quality control, and reduces the need for secondary verifications. Implementation of this technology in combination with Bruker's new VIP-HESI or DART JumpShot ion sources make a significant difference for drug analysis by addressing the most challenging problems, such as separation of coeluting isomers, better sensitivity, and improved data quality while producing results faster than ever before.

Bruker / IonSense DART – full integration and support of the popular analytical technique

Bruker's acquisition of IonSense brings DART-MS ionization into a new era for toxicology and drug identification. This technology removes time-consuming steps by enabling chromatography-free workflows and enabling analysis at the Point of Need. Full system-level integration with Bruker instruments, software, databases, and seamless application support makes DART a powerful solution for rapid drug analysis.

Chrom Tech, Inc. <u>chromtech.com</u> 952-431-6000 Jessica McParland, Product Specialist/Marketing Coordinator jessica@chromtech.com

Buy From Chromatography Specialists

Say goodbye to delayed orders and unresponsive vendors that delay your projects and jeopardize your outcomes. Chrom Tech is a team of chromatography consumable specialists who expedite procurement of the supplies you need. Get back to the work that matters!

- Responsive & Proactive Ordering
- Self-Proclaimed Chromatography Nerds
- Obsessed with Meeting Your Timelines

About Us

Since 1983, Chrom Tech has been dedicated to providing technical expertise in the chromatography field by recommending products to meet your needs. We're proud to be a distributor of chromatography instrumentation and supplies from manufacturers such as Agilent, Restek, Hamilton, Idex, Justrite, and Parker.

Get essentials like vials, well plates, syringe filters, safety waste containment, and GC and HPLC columns. We have chromatography consumables for all your toxicology and forensic needs to help ensure successful analyses in any chromatography application.

What You Can Expect: Get the Answers You Need - Quickly!

We don't like waiting around for answers, so we don't expect you to either. Call Chrom Tech and you'll speak with a real person. Our live chat/email inquiries are answered promptly. We're here to help you solve problems. Whether you know exactly what you want to buy, or have no clue, you can trust that we'll give you a straightforward and honest answer from a real person. Tell us what you're trying to accomplish, and we'll tell you what to buy.

College of American Pathologists

325 Waukegan Road Northfield, IL, US, 60093-2750 sales@cap.org Website https://www.cap.org/ Twitter https://twitter.com/Pathologists Facebook https://www.facebook.com/capathologists LinkedIn https://www.linkedin.com/company/college-of-american-pathologists-cap-Instagram https://www.instagram.com/cap_pathologists/

As the world's largest organization of board-certified pathologists and leading provider of laboratory accreditation and proficiency testing programs, the College of American Pathologists (CAP) serves patients, pathologists, and the public by fostering and advocating excellence in the practice of pathology and laboratory medicine worldwide. Learn more at CAP. org or https://annualreport.cap.org/ and visit the CAP Newsroom.

FINDEN Kura

https://www.linkedin.com/company/kura-biotech

Contact us to obtain more information, request a free sample, or just ask a burning question: sales@kurabiotech.com

About FINDEN

There is a reason every test is critical. 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 analysis in drug testing simple, fast, and precise. We are enabling laboratories to ensure the best results, a more accurate and more human service to the patients, while at the same time increasing throughput.

Our latest release - B-One[®] - is a masterpiece, which allows extremely fast, clean, convenient B-glucuronide 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, such as codeine.

Learn more about B-One[®] here.

About KURA BIOTECH

At Kura Biotech, our dedication is grounded in the strong belief and conviction that we can generate a positive impact in the World. We assist laboratories with their most complex challenges and provide scientists with the best enzymes for sample preparation.

Today, Kura Biotech, ISO 9001:2015 certified, is present in over 15 countries and supplies the world's largest laboratories.

Our commitments: Limitless Spirit, Unapologetic Standards, Give Back Will, Human Connections.

Forensic Advantage Brent Smith <u>bsmith3@forensicadvantage.com</u>

TOXICOLOGY BATCH PROCESSING WITH LIMS INTEGRATION

MAXIMIZE LAB EFFICIENCY AND STANDARDIZE REPORTNG

Batch Processing is a web-based portal that links your LIMS directly to your lab equipment. Enjoy the benefit of real-time workflow notifications and increased equipment utilization and elimination of your manual excel processes throughout your lab.

Increased Efficiency – Batch Processing allows you to automatically assign samples from your cases to the machines and then immediately import results back to their respective case files, reducing manual input and the chance for human error.

Customized Workflows – Our team of experts work with each agency to ensure that Batch Processing is constructed to fill the needs of your specific business roles and workflows. We also work with you to determine how best to set up your data collection points based on how you would like them displayed in a worksheet.

Real-Time Awareness – Profile Dashboards are a great place to see notifications for finished batches, batches that need your review, and alerts to batches that are only partially filled. Get the most out of your equipment by ensuring each cycle is always full.

Standardized Reporting - Batch Processing makes reporting easy as they are standardized and compiled automatically

based on your returns. You no longer need to extrapolate data points from your returns and manually construct reports from your findings.

Forensic Advantage was created to address the unique case and evidence management requirements of local, state, and federal forensic laboratories, law enforcement agencies and medical examiner/coroners. The FAS product suite includes LIMS, Forensic Advantage-Medical Examiner, AuthXAccess, Property Connect, Breath Alcohol Database (BrAD), DNA Databank, and PERK Tracker. In addition to the existing application suite, we continue to innovate and extend the positive affect technology can make on behalf of law enforcement, forensic laboratories, and medical examiners globally.

GenTech Scientific LLC

Contact us today at sales@gentechscientific.com | https://gentechscientific.com/| 585.492.1068

Since 1996, GenTech Scientific has been a leading full solutions provider in refurbished GC, MS, HPLC, GC-MS & LC-MS. Our skilled technicians meticulously inspect, refurbish, and test all our products to precise specifications before they are deemed GenTech Certified. Our instruments come with warranty options to fit your needs. Extend your peace of mind with our multi-vendor service, repair, telephone support, remote diagnostics, installation, and customized training. We provide the options you need to equip your lab for less!

High Sensitivity - Low Cost

When you need maximum sensitivity, the Q-TOF LC/MS system is the ideal high-resolution system. GenTech Scientific's quality refurbished instruments, including warranty and service options offer a low-cost solution. Visit Booth 314 for more information on our featured product!

Grenova

Elizabeth Venafro <u>evenafro@grenovasolutions.com</u> <u>https://grenovasolutions.com/</u>

Grenova's mission is to greatly reduce single-use plastics in laboratories. Our environmental and economic impact on the life sciences industry since 2015:

- 4,581 metric tons in Carbon Emission (CO2e) reduction
- 2,650,848 lbs. of biohazard plastic waste reduction
- \$84,135,258 in savings and cost reduction

Grenova is an innovative company that is revolutionizing lab consumables through high-quality waste reduction solutions. Our existing products include the TipNovus[™] family of pipette tip-washing solutions for labs such as the Automated TipNovus (ATN) Package, a fully integrated tip-washing solution; the TipLumis[™] family of HEPA-filtered, temperature-controlled, UV-sanitized tip storage solutions; GrenoClean, a proprietary and scientifically validated cleaning solution; and Purus by Grenova, a novel microwell plate cleaner launching in 2022. For more information, visit GrenovaSolutions.com.

Integrated Micro Chromatography Systems

Inquiries@imcstips.com +1 (888) 560-2073

Integrated Micro-Chromatography Systems, Inc. (IMCS) is a privately-held biotechnology company that designs, manufactures, and distributes biological reagents from next-generation recombinant proteins to micro-chromatography consumables. The company's two product lines, IMCSzyme[®] and IMCStips[®], serve various clients, including clinical and forensic toxicology labs, academic research facilities, US federal government agencies, and life science companies around the world.

IMCSzyme[®], the first genetically modified, industry-disrupting β -glucuronidase enzyme, is designed to maximize the testing capabilities of laboratories by reducing hydrolysis times and increasing sample processing efficiency. The new IMCSzyme[®] RT is a second-generation β -glucuronidase formulated to hydrolyze drugs of abuse at room temperature in 15 minutes or less. It is the only purified β -glucuronidase enzyme in the market resistant to naturally occurring inhibitors in human urine samples. Learn how to reduce your sample costs while improving quality by contacting us <u>here</u> or emailing <u>inquiries@imcstips.com</u>.

IMCS was recently awarded <u>NIH SBIR and STTR funds</u> to leverage the company's advanced manufacturing and research capabilities and expand the synthesis of glycosphingolipids and sialoglycans. This expansion provides IMCS with new product lines and critically needed biological reagents previously unavailable to the scientific community.

IMCStips[®] are advanced micro-chromatography products centered around patented dispersive solid-phase extraction technology for faster biochemical purification processes. Academic laboratories and life science companies utilize IMC-Stips for purifying new proteins, antibodies, and enzymes, as part of drug target discovery and validation, including the development, screening, and manufacturing of therapeutics.

Indigo BioAutomation

Indigo BioAutomation is an established leader in software automation for the applied and health sciences. Indigo's systems provide laboratory collaboration support as well as analytics. Indigo's flagship system, ASCENT has helped accelerate the review of tens of millions of samples, by automating the processing, reviewing, and reporting of GC/LC-MS results.

ASCENT gives laboratories utilizing chromatography and mass spectrometry a dramatic and innovative new way to improve and grow. Proven and trusted by the nation's leading clinical, toxicology and reference laboratories, ASCENT is a statistically rigorous, automated approach to the processing, reviewing, and report of quantitative date.

Simply put, ASCENT elevates your business performance and technical efficiency.

INTEGRA Biosciences

Contact Name: Customer Service Contact Email: <u>orders-us@integra-biosciences.com</u> Website: <u>www.integra-biosciences.com</u>

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, 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 <u>www.integra-biosciences.com</u> to see our full line of innovative products.

J.G. Finneran Associates

3600 Reilly Court Vineland, NJ 08360 856-696-3605 info@jgfinneran.com www.jgfinneran.com

J.G. Finneran Associates – a leading USA manufacturer of glass and plastic Chromatography vials, closures, septa, microplates and accessories for GC, LC/MS sample preparation and high throughput screening. 236 Chromatography products include crimp and screw thread vials and closures, Snap Ring[™] and Snap Seal[™] vials, patented Poly Crimp[™] and Snap Top Cap[™] closures, headspace and dram vials, limited volume inserts and septa. Microplates includes our patented Multi-Tier[™] plate using vials, deep well sample storage plates, blowdown evaporators, vacuum manifolds, and heat sealing equipment. In additional to our chromatography and sample preparation, Finneran Porvair manufactures Vyon[®] Porous Plastics for life science and medical technologies.

Inquire about our Forensic Sample Packs.

Please visit our website www.jgfinneran.com for complete information on our entire product offerings.

Lipomed Inc. 150 Cambridge Park Drive, Suite 705 Cambridge, MA 02140 Phone: 617-577-7222/ Fax: 617-577-1776/ Website: https://www.lipomed-usa.com Contact Person: Becky Dewey/ <u>ussales@lipomed.com</u>

Lipomed is a leading manufacturer and supplier of high-quality certified reference materials that are developed in-house at our Swiss Facility under ISO 17034 and ISO/IEC 17025 accreditations. Our offering of native and stable isotope labeled materials for mass spectrometry and other analytical techniques are available as ready-to-use (RTU) solutions and bulk powders to provide additional flexibility in choosing the most appropriate form for your testing needs.

Currently, Lipomed Reference Standards include the following classes of substances, their deuterated analogs, and metabolites:

Alcohols & Ethanol Biomarkers Amphetamines & Sympathomimetic amines Antidepressants Antipsychotics Barbiturates Benzodiazepines Cannabinoids Cathinones Cocaines Hallucinogens Opioids Other CNS Depressants Steroids

Lipomed reference materials are available worldwide through our offices in Europe and the USA and a selected network of appointed distributors for local customers. For more information about Lipomed Reference Standards, please visit https://www.lipomed-usa.com

MilliporeSigma

Leanne Dagavarian leanne.dagavarian@milliporesigma.com www.milliporesigma.com

MilliporeSigma is the North America life science business of Merck KGaA, Darmstadt, Germany. With 19,000 employees and 72 manufacturing sites worldwide,our portfolio spans more than 300,000 products enabling scientific discovery. MilliporeSigma brings together the portfolios and expertise of EMD Millipore, Sigma-Aldrich, Supelco, and Cerilliant to provide comprehensive products and support for clinical and forensic laboratories' testing needs.

Neogen

inform@NEOGEN.com NEOGEN.com

Neogen[®] has supplied the Forensic Toxicology industry for more than 30 years and has become a trusted partner for drug screening solutions. We offer a wide range of ELISA tests to provide solutions for drugs of abuse, including designer drugs and emerging drugs. Our solutions ensure confidence in your testing and provide reliable results.

Our comprehensive line of drug detection assays includes more than 115 ELISA kits that can be used to screen more than 300 drugs and their metabolites in various forensic matrices, including oral fluid, whole blood, urine, serum, plasma, meconium, and others. Our testing solutions are designed for high throughput demands, so you can count on a cost-effective, optimized workflow without sacrificing accuracy or time.

Our team of experts is available to address your needs and figure out how we can best support you. For more information, visit our website at <u>NEOGEN.com</u> or email <u>inform@NEOGEN.com</u>

OraSure Technologies

Website: <u>www.orasure.com</u> Contact Name: Christina Werner, National Accounts Manager-Criminal Justice & Forensic Toxicology Contact Email: <u>cwerner@orasure.com</u>

OraSure Technologies manufactures industry specific oral fluid devices in conjunction with other innovative technologies designed to detect or diagnose critical medical conditions. Our diverse product lines include rapid tests for HIV, HCV and COVID-19 antibodies and antigens, multiple testing solutions for detecting drugs of abuse in various sample matrixes, and numerous oral fluid sample collection methods that include stabilization and sample preparation products for molecular diagnostic applications.

Peak Scientific

Contact: Jason Henry Email: <u>jhenry@peakscintific.com</u> Website: <u>www.peakscientific.com</u>

Peak Scientific is a leading innovator in the design, manufacture and

An on-site gas generator from Peak Scientific is the practical and cost-effective alternative to pressurized cylinders. Traditional sources of gas incur on-going delivery, administrative and rental costs, all of which impact on business revenue or facility budgets.

Our forte is in providing our customers with unrivalled peace of mind and hassle free; high quality, dependable gas generation solutions tailored to meet their specific laboratory demands. This comes backed up by our world-class technical support and on-going 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.

Phytronix Technologies Inc.

Contact Name: Melha Yefsah Contact Email: <u>m.yefsah@phytronix.com</u> Website link : <u>www.phytronix.com</u>

The leader in quantitative ultra-fast high-throughput analysis solution for mass spectrometry presents the Luxon Ion Source[®]. This sample introduction system and ionization source guarantees a high throughput, robust and unique solution to an ever-growing number of samples to be analyzed daily. The Luxon Ion Source[®], based on the patented LDTD technology, is now completed with the Azeo Liquid Handler, which offers the opportunity to have a complete automated workflow solution for a wide range of applications.

SCIEX Crystal Holt <u>Crystal.Holt@sciex.com</u> www.sciex.com/applications/forensics-testing

SCIEX offers 50 years of expertise in LC-MS/MS technology combined with a comprehensive portfolio 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.

www.sciex.com/applications/forensics-testing

Sciteck

NEW PRODUCTS: Synthetic UrineCheck[™] test strips and liquid automated analyzer reagents for the detection of synthetic urine submitted for drug of abuse testing and approved neat Oral Split Collector for EIA drugs screening and confirmation with a full line of Neat Oral Fluid Adulteration assays for IgG, Albumin, Oral Enzyme and specific gravity. SCITECK[®] is a full-service company that includes Diagnostics, OEM, R&D and Clinical Reference Laboratory divisions that provide a full range of diagnostic products and healthcare services. Sciteck develops, patents and markets adulteration, biotechnology, urinalysis, clinical chemistry, and toxicology products. Name brand products include AdultaCheck[®] Adulteration Test Strips, Auto UA[®], SVT[™] Urine / Oral Fluid Adulteration reagents, Calibrators & Controls series that include the SAM-SET[™], SVT[™], UR-N-Control and Multi-Drug Level Controls and Calibrators for Oral Fluid.

Shimadzu Scientific Instruments, Inc.

Contact: Rachel Lieberman Contact email: <u>ralieberman@shimadzu.com</u> <u>www.InvestigateYourLab.com</u>

At Shimadzu, we have the analytical tools necessary for your forensic toxicology laboratory to be accurate, efficient, and confident with your results. Our products, including GC-MS, LC-MS/MS, UHPLC and ICP-MS, cover anything from sample preparation to screening, identification and confirmation. In addition, our wide range of instrumentation can be used with a variety of sample types, such as whole blood, urine, plasma, oral fluids, postmortem, tissues, etc.

Siemens Healthineers 511 Benedict Avenue Tarrytown, NY 10591 https://www.siemens-healthineers.com/en-us/drug-testing-diagnostics LinkedIn: https://www.linkedin.com/company/siemens-healthineers/ Twitter: https://twitter.com/SiemensHealthineers/ Facebook: https://www.facebook.com/SiemensHealthineers/

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. 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.

SteelFusion Clinical Toxicology Laboratory, LLC

1103 Donner Ave. Monessen, PA 15062 (P) 724.691.0263 www.steelfusionlabs.com

"Put Your Toxicology Where Your Mouth Is" SteelFusion Clinical Toxicology Laboratory, LLC is a woman-owned, ISO-registered, clinical, forensic, and veterinary toxicology laboratory providing a distinct method of toxicology testing utilizing ORAL FLUID. There exists a growing need for a fast, sensitive, and less-invasive collection method to conduct forensic toxicology than the current standard. Our innovative, break-through methodology empowers customers to make **RAPID** decisions by receiving quantitative results within 48 hours. Over the past seven years, oral fluid has proven to be a reliable method for the detection and quantification of drugs in post-mortem decedents and animals, leading to four U.S. Patents (with one pending) and a Certificate of Grant and European Patent into twenty-eight countries.

Who Benefits? Oral fluid drug testing provides an economical, time- and process-efficient, safe, and non-invasive collection method for medical examiners, coroners, pathologists, clinicians, veterinarians, law enforcement, courts of law and attorneys. It is especially useful when other matrices are limited, difficult, or impossible to collect due to body condition or putrefaction, such as cases involving victims of overdose, suicide, drowning, fire, stillbirth, accidental embalmment, or decomposition. Additionally, our post-mortem oral fluid toxicology reports have been submitted as evidentiary evidence in multiple criminal court cases that have led to guilty pleas, sentencing, and the removal of children from perilous home-steads.

RAPID SERVICES

- Forensic Testing (Criminal Investigation/Drug Paraphernalia/Illicit Drugs/Post-mortem)
- Clinical Testing (Therapeutic Drug Analysis & Monitoring)
- Expert Opinions and Consulting Services
- Court Testimony and Depositions

WHO WE SERVE

- Pre-employment/Employment
- Law Enforcement
- Drug Courts (Adult Probation/CYS/Prison Systems)
- Health Care Providers
- Medical Examiners, Coroners, and Pathologists
- Rehabilitation Facilities
- Veterinarians

"Saliva Technology Excellence with Expert Leadership FUSING old ideologies with new!"

TECAN

14180 Live Oak Ave Baldwin Park, CA 91706 United States T +1 626 373 1530 M +1 626 422 4052 shang.tsai@tecan.com, www.tecan.com

Tecan is a leading global provider of laboratory instruments and solutions in biopharmaceuticals, research, forensics, and clinical diagnostics. Tecan SP specializes in the development, production, distribution and support of automat-ed Positive Pressure Sample preparation instrumentations, providing robust automated workflow solutions for laboratories in the life sciences sector along a vast portfolio of SPE and other sample prep consumables. Had enough of tedious mass spectrome-

try (MS) sample preparation? Despite recent advances in MS instrumentation and detection methodology, its unglamorous counterpart, sample preparation, has not enjoyed the same rate of development. MS sample prep has become a major bottleneck and has hindered the uptake of MS innovation in the life science industry. To help overcome this, Tecan offers the Resolvex A200 positive pressure instrument to improve throughput and reduce human intervention. Our innovative next generation NBE format is not only automation friendly through our unique air lock design. While offering a unique reaction reservoir for on column incubation, hydrolysis, and precipitation prior to SPE extractions.

Thermo Fisher Scientific

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 in forensics, clinical research, and sports anti-doping 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.

Through these and other service brands – Thermo Scientific, Applied Biosystems, Invitrogen, Fisher Scientific and Unity Lab Services – we offer an unmatched combination of innovative technologies, purchasing convenience and comprehensive services. For more information, please visit <u>www.thermofisher.com/forensics</u>

United Chemical Technologies (UCT) 2731 Bartram Rd. Bristol, PA 19007 Phone: <u>215-781-9255</u> / <u>www.unitedchem.com</u> Contact Person: Bethany Magrann / <u>bmagrann@unitedchem.com</u>

UCT, LLC is a vertically integrated manufacturer of high-quality sample prep products and rugged U/HPLC columns utilized in forensic applications. Continuously striving to streamline the extraction process, UCT offers a wide selection of Solid Phase Extraction (SPE) phases, convenient Select[®] pH buffer pouches, Micro-Prep[™] micro-elution plates, Refine[®] ultra-filtration plates and columns, SELECTRA-SIL[®] high purity derivatizing reagents, positive pressure/glass-block manifolds, SPeVAP[®] solvent evaporator, GC Liners, Selectra[®] U/HPLC columns, Selectrazyme[®] β-glucuronidase and Abalonase[™] purified β-glucuronidase enzymes. We are excited to present our new SelectraCore[®] product line of core-shell HPLC columns that offer superior performance in drugs of abuse analysis.

Visit <u>www.unitedchem.com</u> to find the most comprehensive forensic applications developed by our applications Specialists in addition to our entire listing of available product offerings.

UTAK Laboratories, Inc. Contact: Christina Plutchak chritina@utak.com www.utak.com Tier II Sponsorship

At UTAK, we're proud to call ourselves "control freaks", but not in the way you might think.

That's because our obsession lies not in taking control but in giving control—to the testing labs that need the finest quality control materials for their clinical and forensic toxicology test methods. Our close-knit group crafts the quality controls these labs depend upon for every kind of analysis, including a wide range of comprehensive stock controls in urine, serum, blood, oral fluid and more, as well as starting matrices for laboratories seeking to develop in-house quality control material. We also create personalized control solutions to support the new methods these labs develop. Our dedication is grounded in our belief that better control for testing labs leads to more accurate results and ultimately, to better safeguarding of health and safety standards.





Providing Excellence in Data Quality

Shimadzu's Liquid Chromatograph Mass Spectrometers (LC-MS, LC-MS/MS, Q-TOF) provide high performance and excellence in data quality, enabling dramatic improvements and allowing sensitive quantitative analyses with exceptional robustness and ease of use.

Enhanced performance Sensitivity and Robustness

Shimadzu's most powerful tandem mass spectrometer, the LCMS-8060NX utilizes proprietary technologies and more efficient ion transfer to achieve outstanding speed and sensitivity, while robust operation results in maximized uptime, the utmost in data quality, and a more efficient analytical workflow.

Effortless performance Perform Critical Analysis with Confidence

The research-grade LCMS-9030 Q-TOF LCMS combines the engineering DNA from our proven LC-MS/MS platform with powerful new TOF architecture to transform high mass accuracy workflows, resulting in high-resolution, accurate-mass detection with incredibly fast data acquisition rates.

Learn more about Shimadzu's LC-MS systems. Call (800) 477-1227 or visit us online at **www.ssi.shimadzu.com** Shimadzu Scientific Instruments Inc., 7102 Riverwood Dr., Columbia, MD 21046, USA



Sciteck Liquid Adulteration Reagents and NIST Traceable Control and Calibrators Sciteck's On-Site SVT Products and Controls

Adulta Check™4, 6, and 10



Urine Luck Check Strips"

Synthetic UrineCheck**

the inte





Essential in Labs

Alternative Biomedical Solutions

Toll Free: (866) 895-1388 Website: <u>www.altbio.com</u> Email: <u>sales@altbio.com</u>

Exceptional lab equipment



Impartial consulting



Highly responsive, world-class technical service



UHR-TOF MS TARGETSCREENER 4D

A Comprehensive Solution for Screening and Quantification powered by Trapped Ion Mobility

- Curated exact mass database with RTs, up to 10 diagnostic ions and CCS values
- Comprehensive reliable screening and ultra-sensitive quantification
- Updateable database allowing retrospective analysis of forensic and toxicological relevant compounds



For more information please visit www.bruker.com



TIMS-OTOF MS TIMSTOF PRO 2

The new standard for high speed, high sensitivity 4D-Multiomics

Powered by PASEF

- Dual TIMS technology delivers near 100% duty cycle
- PASEF provides unrivaled sensitivity, specificity and speed
- Unlock CCS-enabled workflows for confident identification

For more information please visit www.bruker.com

For Research Use Only. Not for use in clinical diagnostic procedures.

Testing Too Complicated?





Keep It Simple With Neogen[®] Visit our team at booth #505





Learn more about our trusted solutions from our forensic team. ELISA Drug Detection | Oral Fluid Collection | Automated Equipment

© Neogen Corporation, 2022. Neogen is a registered trademark of Neogen Corporation.

Let's Talk Tox "PUT YOUR TOXICOLOGY WHERE YOUR MOUTH IS"



MATRIX	EASY & SAFE COLLECTION	RAPID RESULTS	ACCOLADES
Oral Cavity Fluid (1 mL)	Non-Invasive Collection (Within 15 min.)	Quantitative Results (Within 48 hrs.)	ISO 17025 ISO 15189 4 U.S. Patents 1 E.U. Patent



steelfusionlabs.com

1103 Donner Avenue Monessen PA 15062

724.691.0263

RAPID SERVICES

- Forensic Testing
- Post-mortem
- Drug Paraphernalia, Illicit Drugs
- Clinical Testing (Therapeutic Drug Analysis & Monitoring)
- Expert Opinions and Consulting Services
- Court Testimony

WHO WE SERVE

- Employment
- Law Enforcement
- Drug Courts (Adult Probation/CYS/Prison Systems)
- Health Care Providers
- Medical Examiners, Coroners, and Pathologists
- Rehabilitation Facilities
- Veterinarian

"Saliva Technology Excellence with Expert Leadership FUSING old ideologies with new! VISIT US AT BOOTH #630



WHEN RESULTS MATTER



Technical Support methods@unitedchem.com



Û C I

-

UCT

10P

SPeVAP

