The Qualitative Analysis of Drug of Abuse by Ultra High-Pressure Liquid Chromatography Tandem Mass Spectrometry (UHPLC-MS/MS) in Human Urine

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Introduction

One of the leading causes of injury-related death in America is drug overdoses, with many attributed to polysubstance abuse.[1] To combat the national overdose crisis, the CDC has developed the Overdose Data to Action (OD2A) project to compile data on trends and characteristics for nonfatal drug overdoses across the country. The data will then be utilized to develop preventative strategies on a state and local level through initiatives such as linkage to care, health interventions and harm reduction programs.[2] The New Jersey Public Health & Environmental Laboratories (NJPHEL) is developing a qualitative method for testing drugs of abuse by Ultra High-Performance Liquid Chromatography-Tandem Mass Spectrometry (UHPLC-MS/MS) in human urine. The data collected through this method will provide the CDC and participating hospitals with further information surrounding the overdose epidemic in New Jersey. Publications involving the use of LC-MS/MS to identify psychoactive substances in urine were used as references.[3,4,5] The method identifies 59 substances recommended by the CDC and experts; including opioids, stimulants, benzodiazepines, antidepressants, cannabinoids, and hallucinogens in remnant urine samples from patients admitted to hospitals for nonfatal overdoses. The overall range of the method will be validated between 5 ng/mL and 250 ng/mL. The analytical cutoff for the method is set at approximately 5 ng/mL, which is five times the signal/noise ratio. Validation for this method will be established by analyte separation via liquid chromatography, the optimization of each analyte, and the establishment of sensitivity and reporting thresholds. Once the method is validated the NJ Chemical Terrorism (CT) lab will receive specimens from two participating hospitals for testing.

Methodology

Sample Preparation

Sample preparation is done by aliquoting 50 µL of the specimen and 50 µL of methanol into a 0.5 mL centrifuge tube. The samples are vortexed and centrifuged at 14,000g for 10 minutes. Then 40 µL of the supernatant is transferred into a vial with 140 µL of 50:50 MeOH/Water and 0.01% FA solution, and 20 µL of surrogate for injection. After vortexing, 2.0 µL of the sample is injected by the instrument for testing, producing a 10x dilution of the analytes

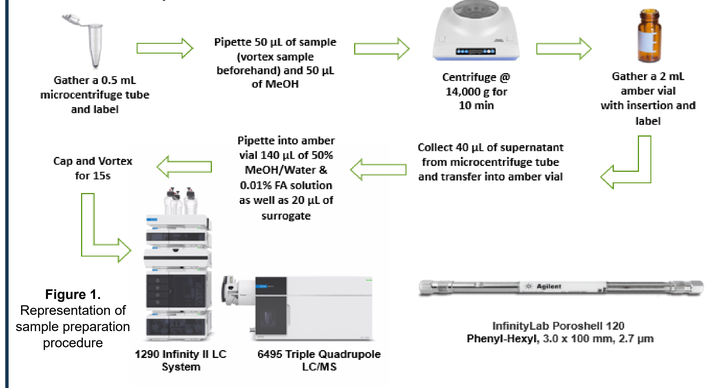

Figure 1. Representation of sample preparation procedure

Method Parameters	
System	Agilent 1290 UHPLC - Triple Quadrupole 6495C MS/MS
Software	MassHunter Workstation for LC/TQ (Version 10.1)
Analytical Column	Agilent InfinityLab Poroshell 120 Phenyl-Hexyl, 3.0 x 100 mm, 2.7 µm (685975-312)
Column Temperature	50˚ C
Mobile Phase A	Water, 0.01% formic acid & 0.315g of ammonium formate
Mobile Phase B	Methanol & 0.01% of formic acid
Run Time	14 min
Injection Volume	2.0 µL
Seal Wash	80:20 OP water/IPA

Table 1. LC Parameters for the HPLC-MS/MS method

Gradient Program				
Time (min)	A [%]	B [%]	Flow [mL/min]	Max. Pressure Limit [bar]
0.00	70.00	30.00	0.350	600.00
2.00	65.00	35.00	0.350	600.00
3.00	63.00	37.00	0.350	600.00
4.50	60.00	40.00	0.350	600.00
5.00	57.00	43.00	0.350	600.00
6.00	53.00	47.00	0.350	600.00
8.00	53.00	47.00	0.350	600.00
12.00	0.00	100.00	0.350	600.00
14.00	0.00	100.00	0.350	600.00

Table 2. Gradient program in LC method

Results

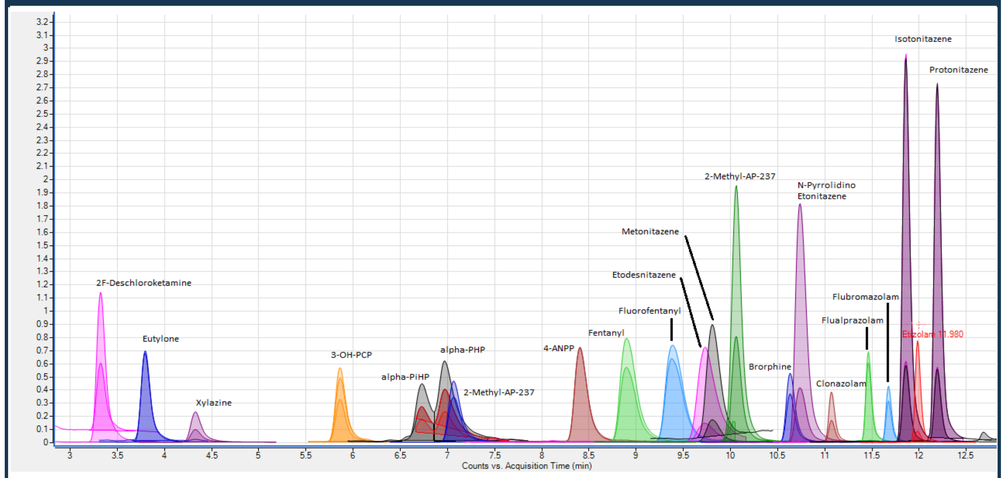

Figure 2. Extracted ion chromatogram of selected analytes from the method under MRM

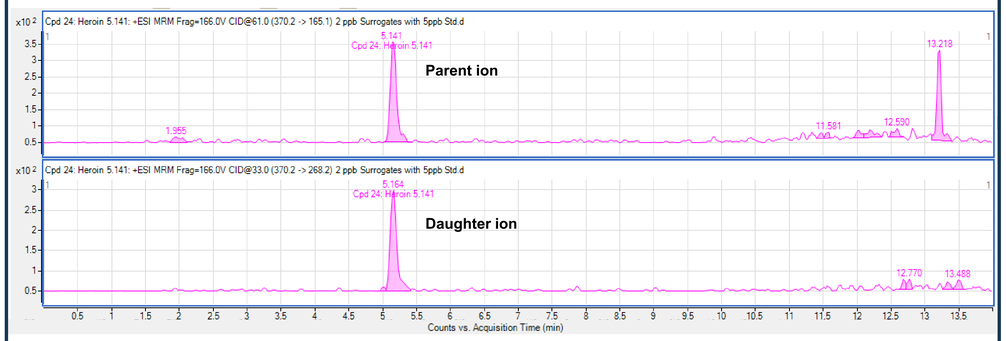

Figure 3. Chromatographic representation of a single analyte, in a urine matrix, displaying its retention time, precursor ion and product ion

Class	Substance	Retention Times	Precursor Ion	Product Ions		Class	Substance	Retention Times	Precursor Ion	Product Ions	
				Primary Qualitative	Secondary Qualitative					Primary Qualitative	Secondary Qualitative
Novel Stimulant/Hallucinogen	2F-Deschloroketamine	3.407	222.1	109.1	163.1	Opioid	Carfentanil	10.17	395.0	113.0	105.0
Opioid	2-Methyl-AP-237	10.20	287.2	117.1	115.0	Benzodiazepine	Clonazepam	11.36	316.1	270.1	214.1
Novel Stimulant/Hallucinogen	3-HO-PCP *	5.962	260.2	86.10	107.1	Benzodiazepine	Clonazolam	11.07	354.0	308.0	280.0
Opioid	4-ANPP	8.474	281.0	188.0	105.0	Stimulant	Cocaethylene	7.300	318.2	196.1	82.10
Novel Stimulant/Hallucinogen	4-HO-PCP *	5.961	260.2	86.10	107.1	Stimulant	Cocaine	5.733	304.2	182.2	77.10
Opioid	6-Acetylmorphine	2.565	328.2	211.1	193.0	Opioid	Codeine	2.323	300.2	152.1	115.1
Opioid	Acetylfentanyl	7.120	323.0	105.0	188.0	Cannabinoid	Delta-8 (THC Isomer)**	13.42	315.2	193.1	123.1
Novel Stimulant/Hallucinogen	alpha-PHP	6.816	246.2	77.10	105.1	Cannabinoid	Delta-10 (THC Isomer) **	13.42	315.2	193.1	123.1
Novel Stimulant/Hallucinogen	alpha-PiHP	7.062	246.2	77.10	91.10	Benzodiazepine	Diazepam	12.33	285.0	257.0	228.0
Benzodiazepine	Alprazolam	11.75	309.1	281.1	205.1	Benzodiazepine	Etizolam	11.90	343.1	314.0	310.0
Stimulant	Amphetamine	2.759	136.1	119.2	91.10	Opioid	Etodesnitazene	9.832	352.2	100.2	72.20
Stimulant	Benzoylecgonine	4.254	290.1	168.1	105.0	Novel Stimulant/Hallucinogen	Eutylone	3.877	236.1	218.2	188.1
Benzodiazepine	Bromazolam	11.94	353.0	205.1	325.0	Opioid	Fentanyl	8.981	337.2	105.1	188.1
Opioid	Brorphine	10.64	400.1	104.1	182.9	Benzodiazepine	Flualprazolam	11.43	327.0	223.0	299.0
Opioid	Buprenorphine	10.75	468.3	414.2	396.2	Benzodiazepine	Flubromazolam	11.60	371.0	223.0	292.1
Opioid	Fluorofentanyl	9.472	355.3	206.2	123.1	Opioid Antagonist	Naloxone	2.228	328.2	212.1	268.1
Other Substance	Gabapentin	2.265	172.1	137.1	154.2	Opioid	Norbuprenorphine	7.562	414.3	101.1	83.10
Opioid	Heroin	5.141	370.2	165.1	268.2	Opioid	Norfentanyl	4.214	233.2	84.10	55.10
Opioid	Hydrocodone	2.817	300.2	199.1	128.2	Opioid	N-Pyrrolidine Etonitazene	10.73	395.2	98.10	56.10
Opioid	Hydromorphone	1.764	286.2	185.1	157.1	Benzodiazepine	Oxazepam	11.55	287.1	241.1	269.1
Opioid	Isotonitazene	11.78	411.2	100.1	72.10	Opioid	Oxycodone	2.679	316.2	298.2	241.1
Benzodiazepine	Lorazepam	11.41	321.0	275.0	229.1	Opioid	Oxymorphone	1.695	302.1	284.2	227.1
Stimulant	MDA	2.960	180.1	163.1	135.1	Opioid	Protonitazene	12.10	411.2	100.1	72.20
Stimulant	MDEA	3.677	208.1	163.1	105.1	Benzodiazepine	Temazepam	11.93	301.1	255.1	177.1
Stimulant	MDMA	3.184	194.1	163.1	135.1	Cannabinoid	THC-O	13.71	357.3	315.2	193.1
Opioid	Methadone	11.45	310.2	105.1	265.2	Cannabinoid	THC-P	13.58	343.3	221.1	123.1
Stimulant	Methamphetamine	2.983	150.1	91.10	65.10	Antidepressant	Tianeptine	11.47	437.1	292.0	228.1
Opioid	Metonitazene	9.875	383.2	72.20	100.2	Opioid	Tramadol	4.772	264.2	58.10	n/a
Opioid	Morphine	1.652	286.2	201.1	165.1	Other Substance	Xylazine	4.417	221.1	89.70	163.8
Novel Stimulant/Hallucinogen	N,N-Dimethyl Pentylone	5.087	250.1	100.1	175.1						

Table 3. Experimental data of the retention times, precursor ions and product ions for all 59 substances tested in the method, excluding surrogates

*/ **Analytes are co-eluting and are measured together

Conclusions

- After several assessments, the sample preparation displayed in **Figure 1** was determined to be highly effective as it provided results with minimal background noise. Surrogates were selected based on molecular makeup and are used as a quality control for the specimens and the instrument.
- The analytes were adequately separated by the UHPLC instrument. Twenty selected analytes are represented in **Figure 2** as a reference. All analytes are distinguished by baseline separation or by m/z ratio and detected at levels of 5 ng/mL.
- Data analysis was conducted to establish peak separation of each target compound as displayed in **Figure 3,** for verification of the analytes.
- Retention times and precursor/product ion pairs were established along with selected precursor to product transitions to allow for optimal detection, as shown in **Table 3.**
- The CT laboratory is currently validating a standard operating procedure for the method. Once approved we will begin receiving specimens from two participating hospitals for testing in the New Jersey area.
- The data collected through this method will provide the CDC, participating hospitals, and local governments with additional insights related to biosurveillance surrounding the overdose crisis.
- As needed, the CT laboratory will continue to incorporate or exclude substances from the method based on recommendations from the CDC and medical experts

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