Identification and Prevalence Determination of Novel Recreational Drugs and Discovery of Their Metabolites in Blood, Urine, and Oral Fluid

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Abstract

Designer drug products which contain a variety of unregulated psychoactive constituents have become mainstream on the illicit drug market. These compounds, collectively known as novel psychoactive substances (NPS) are generally abused for their stimulatory and euphoric effects. Because of their physical and mind-altering effects, NPS are commonly used at electronic dance music (EDM) festivals to enhance attendees' experience of the music and the event. Their widespread use at EDM festivals has been well documented and several adverse events and fatalities associated with the ingestion of these emerging recreational drugs have been reported in the United States. The diversity and rapid turnover in the prevalence of any particular NPS at any given point of time has created several challenges for public health officials, law enforcement, and forensic science communities.

Over the course of two years, blood, urine and oral fluid samples were collected from EDM festival attendees, in addition to survey data regarding prescription and recreational drug use within the last week, with the aims of discovering emerging NPS, ascertaining their overall prevalence, evaluating the viability of oral fluid as an alternative matrix for drug detection compared to blood, and determining patterns of use and trends, especially for NPS within this population. Rapid changes in the drug market of synthetic compounds frequently cause epidemiological studies to be published long after drugs have cycled through the peak of their popularity with users, and the scope of testing frequently fails to detect, identify or report the most recently available drugs. Additionally, incomplete literature exists regarding the identity of metabolites of many NPS, making laboratories abilities to maintain a current scope difficult and incomplete. To address this issue, *in vitro* metabolism studies for alpha-pyrrolidinophenone (alpha-PVP), methylone and dimethylone were carried out using human liver microsomes. Metabolites identified using this *in vitro* process were subsequently compared to metabolites produced *in vivo* from authentic human drug user samples described below to determine the extent to which each metabolite could be detected in authentic biological specimens of recreational users and which metabolites would serve as the most valuable biological markers of use.

Over 2014 and 2015, biological samples were collected from 396 individuals (126 blood samples; 227 urine samples; 122 oral fluid samples screened with the Alere DDS2; and 384 oral fluid samples collected with the Immunalysis Quantisal™ oral fluid collector). In survey questions, seventy-two percent of the participants had reported using a recreational drug or medicinal substance within the last week. Users most commonly reported using marijuana and alcohol, which were followed by "Molly" and cocaine. Of the 396 individuals tested, approximately 75% of the population was positive in at least one biological specimen for drugs and/or alcohol. With respect to NPS and/or 3,4-methylenedioxy-methamphetamine (MDMA), 37% of the positive samples were confirmed in at least one biological matrix for one or more NPS and/or MDMA. In 2014, several samples were confirmed for alpha-PVP (n=17), however in 2015 there was not a single positive case for alpha-PVP. Instead, increasing numbers of subjects were positive for the NPS ethylone, which demonstrates and supports the high rates of turnover NPS.

In comparing the three matrices, there was good agreement between the specimens with respect to reporting positive results. The study demonstrated the value of using oral fluid as a specimen for drug detection compared to blood. Finally, through the use of *in vitro* metabolism studies, metabolic pathways for alpha-PVP and dimethylone were proposed. These metabolites were subsequently identified in authentic blood, urine and oral fluid specimens. The most prevalent metabolite for alpha-PVP was the 5-OH-PVP metabolite and for dimethylone were methylated dimethylone in blood and oral fluid and the hydroxylated dimethylone in urine. In a few cases, the parent drug was not confirmed, however, the presence of unique metabolites could be used in many cases to indicate which parent drug the subject had ingested.

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Executive Summary

According to 2013 data from the National Survey on Drug Use and Health, an estimated 24.6 million Americans had used illicit substances within the prior month (1). The emergence of novel psychoactive substances (NPS), which refers to a broad category of products containing unregulated constituents that are readily available, has created a growing trend in young adults as popular substances of abuse (2). Their widespread availability, ease of purchase, and not being included in routine drug testing are cited as part of their overall appeal and increased prevalence (3). In the United States, electronic dance music (EDM) festivals have become a popular venue for these recreational drugs (4-5) with reports of as many as 70% of attendees may be using recreational substances (6-10). Within the last two years, several reports of fatal overdoses and non-fatal drug intoxications have stemmed from NPS use at EDM festivals within the United States.

Although the federal government and states are attempting to regulate these substances, NPS are still readily available to users because they are easily manufactured and manipulated to avoid legislation (11-12). In 2014, the European Monitoring Centre for Drugs and Drug Addiction (EMCDDA) reported an additional 101 NPS drugs found during seizures for the first time (13), which has steadily been increasing since 2005. According to the National Forensic Laboratory Information System (NFLIS), cases reported to contain synthetic cannabinoids and synthetic cathinones dramatically increased between 2010 and 2013 (14). For synthetic cannabinoids, reports increased from 469 in 2010 to 37,500 in 2014, and for synthetic cathinones the increase was from 142 in 2010 to 274,862in 2013 (14).

Inevitably, published methodologies for the detection and identification of NPS lag behind the market itself. Continuous monitoring of drug trends is necessary in order to make sure drug testing panels are targeting relevant compounds. However, because of limitations associated with the lack of confirmatory testing procedures for the newest compounds and the inability of most forensic laboratories to offer updated and comprehensive testing, only a fraction of the problems associated with emerging NPS drugs are recognized and reported. Additionally, there are limited epidemiological studies that examine the prevalence of these compounds and compare these compounds in blood, urine and oral fluid as well as identify their metabolites (15). The collection of paired blood, urine, and oral fluid specimens from authentic recreational users allows for the compilation of valuable information to be obtained from a population at risk for using some of the most novel recreational chemicals on the market.

Oral fluid has become a popular biological matrix for forensic use based on its rapid collection and ability to provide results immediately, allowing for correlation of levels to pharmacodynamic effects (16). Several roadside studies have evaluated commercially available oral fluid screening and provided useful data concerning drug use of random driving populations (16-18). However, they did not evaluate a targeted group of high-prevalence drug users for novel recreational drug use. Additionally, most of the literature regarding the use of oral fluid has compared the use of on-site collection devices and the screening results they generate to compounds confirmed using either serum/plasma or urine (19-20). This data

provides limited correlations between the concentrations in oral fluid compared to the concentrations found in the blood. This study sought to compare oral fluid and blood samples to allow for the correlation of concentrations between compounds detected in these two specimens to be made, strengthening the assessment of oral fluid as a viable biological matrix for forensic use.

Further, metabolic studies for novel drugs remain limited. Current research shows that many of the parent compounds in the NPS category are unstable or extensively metabolized, reinforcing the importance of determining the identity of metabolites and breakdown products in order to detect use of these novel drugs in urine samples (21). Much of the existing research on metabolism of emerging NPS is limited only to *in vitro* studies and while of qualitative value these profiles frequently do not reflect the relative prevalence of metabolites following ingestion *in vivo*, and consequently the most appropriate target compounds for which to develop lab tests (22). With respect to the identification of metabolites in authentic specimens, these investigations have typically been limited to animal specimens or more rarely, human urine. Very few reports have examined the extent of these metabolites in blood samples.

The rapid evolution of the NPS drug market has resulted in limited or delayed information regarding recreational drug use trends and appropriate markers for use in biological specimens. The collection of multiple paired blood, urine and oral fluid samples provided a comprehensive approach for analysis that included: identification of parent drug; the presence of metabolites and their relative prevalence in authentic specimens; ability to investigate the viability of oral fluid a as a noninvasive biological specimen for confirmation of drug ingestion; relative concentrations of NPS in blood compared to oral fluid; investigation of self-reported drug use relative to the prevalence of individual drugs in biological specimens; and overall drug use trends for EDM festival attendees.

Methods

Sample Collection

All research was Institutional Review Board (IRB) reviewed, approved and conducted in full compliance with U.S. Federal Policy for the Protection of Human Subjects (Basic DHHS Policy for Protection of Human Research Subjects; 56 FR 28003). A total of 396 subjects (188 males; 127 females; 81 unidentified) were verbally recruited by peer recruiters for this study at an EDM festival in Miami in the spring of 2014 and 2015. Consenting participants provided demographic information and whether or not they had taken any medication or recreational drugs within the last week. Participants were asked to donate blood (collected by a licensed phlebotomist), urine and oral fluid, but were not required to donate all three specimens. All samples collected at the festivals were initially stored refrigerated (4˚C), and shipped on dry ice, prior to being frozen at the laboratory (-80˚C) until analysis. At the conclusion of each festival, blood and oral fluid samples were shipped overnight The Center (Willow Grove, PA) for analysis, while urine specimens were shipped to the Armed Forces Medical Examiner's Office: Division of Forensic Toxicology (Dover, DE).

Sample Analysis

Initially, all survey data were compiled into an Excel spreadsheet to allow for data management purposes. All biological samples were initially screened for common drugs of abuse, therapeutic compounds, and emerging NPS using a variety of approaches described below. Any sample that screened positive for one or more drugs was sent for confirmatory analysis and quantitation when appropriate. An overview of the biological sample analysis including the location for the analysis and analytical platform for both screening and confirmatory methods is shown in Figure 1.

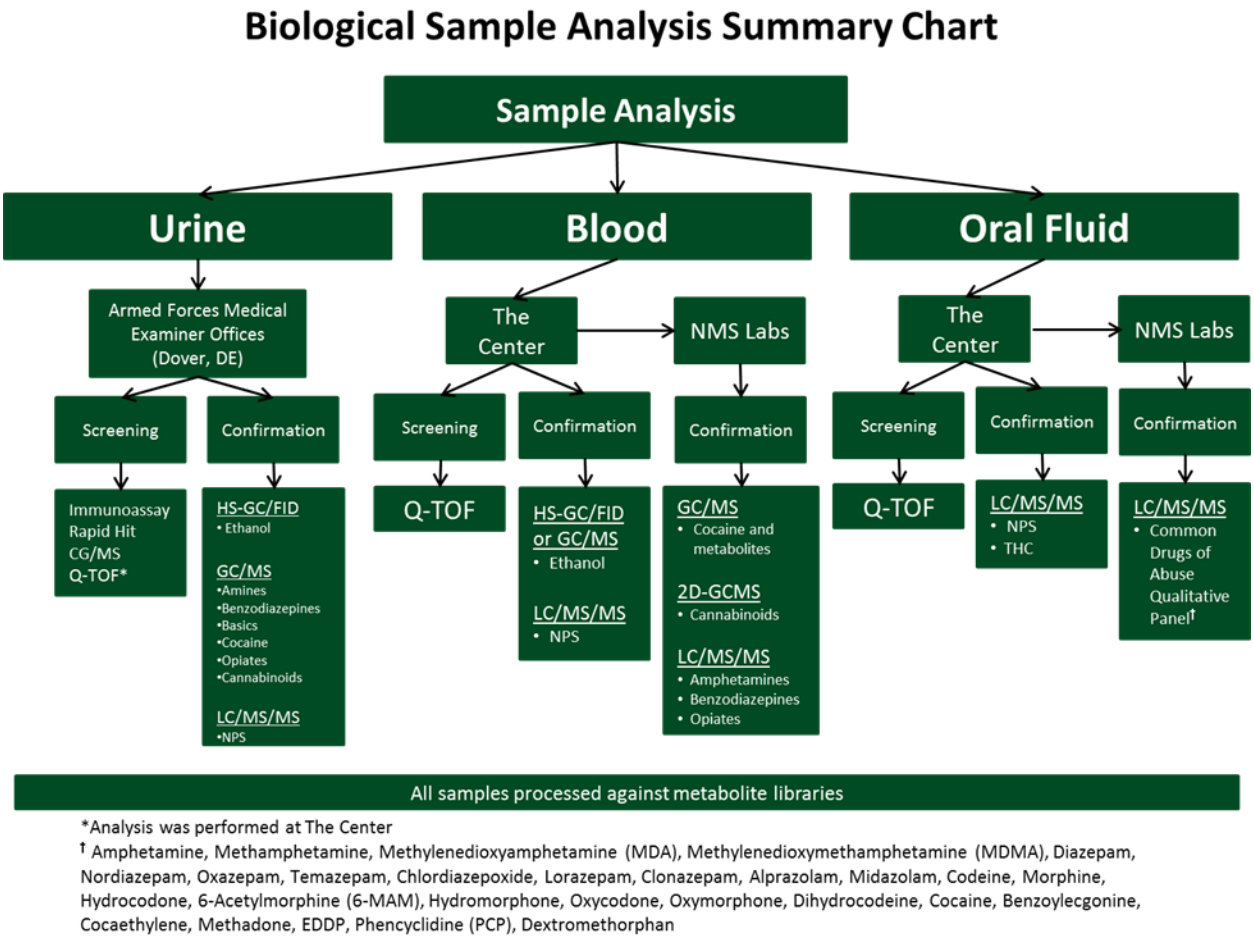

Figure 1 Summary of biological sample analysis by specimen type including location of analysis and analytical platform for screening and confirmatory methods.

Results and Discussion

Survey Data

Survey data was obtained from 342 subjects. A total of 188 males and 127 females provided survey information, with 27 subjects not indicating a gender. The average age of the participants in was 22.5 years old (±5 years). In both 2014 and 2015, 72% of the respondents had reported using a medicinal substance or recreational drug within the past week (Note: In 2014, one person did not answer that question). The most common substance participants

indicated that they had taken was marijuana, followed by alcohol and "Molly". "Molly" is a slang term, which previously has referred to MDMA, however, today the term is most commonly associated with methylone. Shown in Figure 2 are the percentages of responses for the most commonly reported recreational drugs used with the last week.

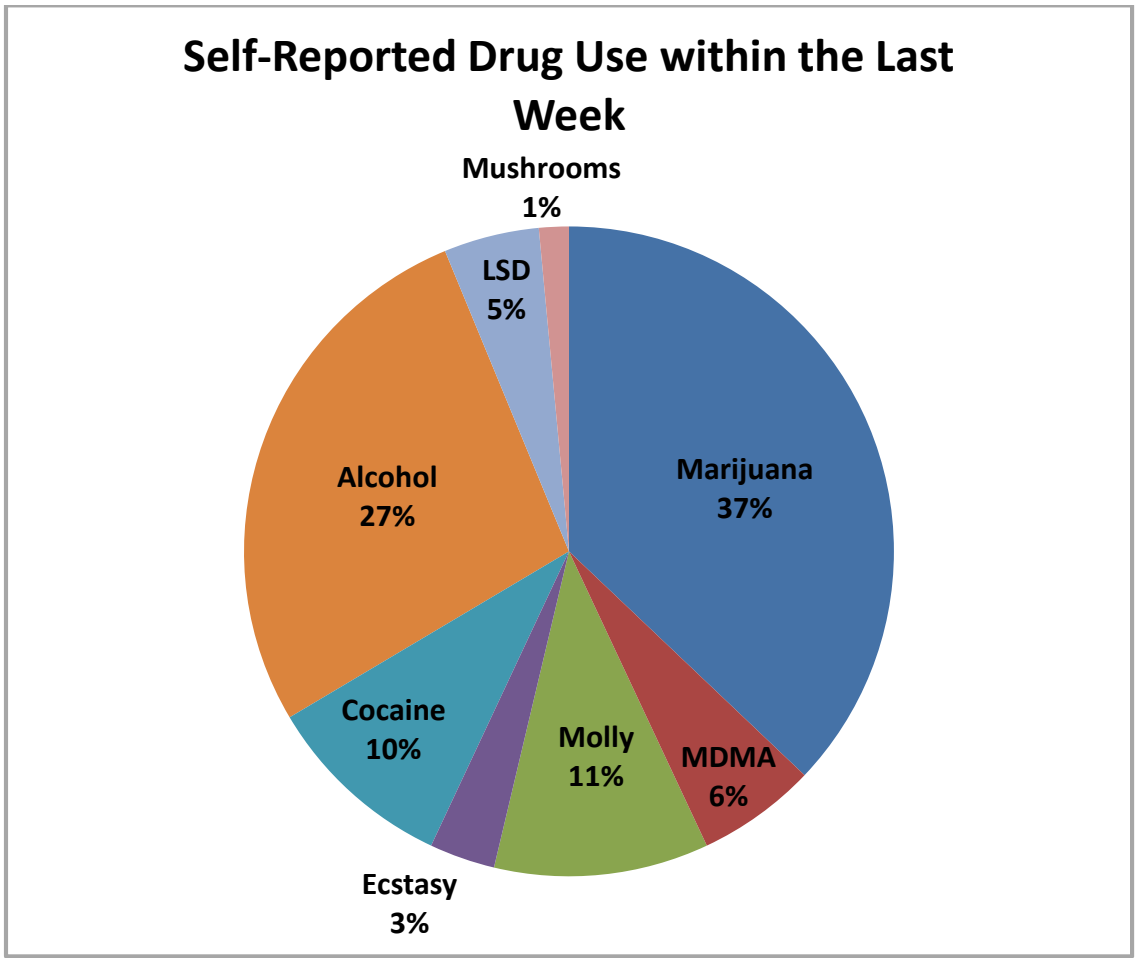

Figure 2 Festival attendees self-reported medicinal or recreational drug use within the last week among users reporting recent drug use.

Blood Samples

Seventy-two percent (72%) of blood cases were positive for at least one drug and/or alcohol. With respect to alcohol, approximately 40% of the population was positive with an average blood alcohol concentration was 102 mg/dL (±66) with a range of 10-304 mg/dL. Sixty-six percent of the samples (n=33) were positive for alcohol only, four samples (8%) were positive for alcohol and more than one drug, and 22% (n=11) were positive for alcohol plus one drug. Excluding blood cases that were positive for alcohol (n=34), 45% of the population for at least one drug in the blood. Sixteen samples confirmed positive for an NPS and/or MDMA in the blood. The primary NPS identified in the blood was methylone (n=9), followed by alpha-PVP (n=6) and dimethylone (n=6). Shown in Table 1 is the number of confirmatory positive results for each drug.

Table 1. Confirmatory results for the number of positive samples by drug in blood.

Class	Analyte	Number of Positive Samples
Benzodiazepines	Alprazolam/α-OH-Alprazolam*	6
	Clonazepam/7-amino-Clonazepam*	1
Amphetamines	MDMA/MDA*	3
	MDMA	3
	Amphetamine	3
	Methamphetamine	1
Cocaine	Cocaine/Benzoylecgonine*	6
	Benzoylecgonine	6
	Cocaine	3
	Cocaethylene	2
Opiates	Oxycodone	2
	Tramadol	1
	Methadone/EDDP*	1
	Hydrocodone	1
	Morphine	1
	Oxymorphone	1
NPS	Methylone	9
	Dimethylone	6
	Alpha-PVP	6
	Ethylone	2
	4-FA	2
	Butylone	1
THC	THC/THC-COOH*	41

*Positive sample contained both parent and metabolite.

Urine Samples

Seventy-nine percent (79%) of the urine samples were positive for at least one drug and/or alcohol. Seventeen percent of the urine samples (n=38) were positive for alcohol only, 12 samples (5%) were positive for alcohol and a single drug and 12% were positive for alcohol and more than one drug. Fifty-one samples confirmed positive for an NPS and/or MDMA in the urine. The primary NPS identified in the urine was methylone (n=24), followed by ethylone (n=19), alpha-PVP (n=12) and dimethylone (n=11). Shown in Table 2 is the number of confirmatory positive results for each drug.

Table 2. Confirmatory results for the number of positive samples by drug in urine.

Class	Analyte	Number of Positive Samples
Benzodiazepines	Alprazolam/α-OH-Alprazolam*	8
	Clonazepam/7-amino-Clonazepam*	1
	Oxazepam	1
Amines	MDMA/MDA*	17
	Amphetamine	10
	Methamphetamine	6
	MDA	4
	MDMA	2
	PMMA	1
Cocaine	Cocaine/Benzoylecgonine*	28
	Benzoylecgonine	17
	Cocaethylene	13
Opiates	Oxycodone	4
	Tramadol	2
	Oxymorphone	2
	Methadone/EDDP*	1
	Morphine	1
	Hydrocodone	1
	Hydromorphone	1
	Dihydrocodeine	1
	Buprenorphine/Norbuprenorphine	1
NPS	Methylone	24
	Ethylone	19
	Alpha-PVP	12
	Dimethylone	11
	Butylone	8
	4-FA	3
	2C-B	1
	2C-I	1
	25-I NBOMe	1
	25-I NBOH	1
Other Compounds	Dextromethorphan	8
	Dehydronorketamine/Norketamine/Ketamine*	5
	PPA	5
	Chlorpheniramine	4
	Fluoxetine	3
	Quetiapine/Norquetiapine	2
	Sertraline/Desmethylsertraline	2

	Doxylamine	2
	Bupropion	1
	Cyclobenzaprine	1
	Methylphenidate	1
	DMAA	1
	Psilocin	1
	Amantadine	1
	Pseudoephedrine	1
	Citalopram	1
	LSD	1
	Phenobarbital	1
	Azacyclonal	1
THC	THC/THC-COOH*	95

*Positive sample contained both parent and metabolite.

Oral Fluid Samples

In 2014, a total of 122 oral fluid samples were screened in the field using the Alere® DDS2 for amines (amphetamine and methamphetamine), benzodiazepines, cannabis, cocaine, and opiates. The results for each respective class are listed in Table 3.

Table 3 Results of the DDS2 screening results relative to the results obtained in the confirmatory oral fluid specimen (n=122).

	Positive	Negative	False Positives	False Negatives	Invalid
Cannabis	27	89	0	3	3
Cocaine	12	107	0	0	3
Amphetamine	3	118	0	0	1
Methamphetamine	1	117	1	0	3
Benzodiazepines	1	120	0	0	1
Opiates	0	119	0	0	3

For cocaine, amphetamine, benzodiazepines and opiates, there were no cases in which a result produced for one of the target drug classes by the device was not confirmed in the laboratory based oral fluid test (i.e. false positive). To determine false negatives, the results of the DDS2 were compared to the results of the additional oral fluid sample generated via LC-QTOF (All oral fluid samples were confirmed for cannabis). This resulted in 100% sensitivity, specificity and accuracy for each of those drug classes. For cannabis, there were three cases where a positive result was produced by the device, but detected in the confirmatory specimen, resulting in 90% sensitivity and 97.4% accuracy. There was one case where a positive result was produced on the device for methamphetamine, but methamphetamine was not detected in the confirmatory specimen. The overall sensitivity, specificity and accuracy of the device were 93.6%, 99.8%, and 99.3%, respectively. Keep in mind however that the device does not test for NPS drugs.

Fifty-two percent (52%) of the oral fluid samples comprehensively tested in the laboratory for common therapeutic, abused and NPS drugs, were confirmed positive for at least one drug within the scope of the confirmatory methods. The majority of the confirmed positive samples were positive for THC (76%), followed by an NPS and/or MDMA (44%). The main NPS confirmed in the oral fluid were ethylone (n=56), methylone (n=24), and alpha-PVP (n=12), although as noted the relative prevalence changed from year one to year two. The number of positive samples by drug for the confirmatory testing results is shown in Table 4.

Table 4. Confirmatory results for the number of positive samples by drug in oral fluid.

Class	Analyte	Number of Positive Samples
Benzodiazepines	Alprazolam/α-OH-Alprazolam*	2
	Lorazepam	2
	Clonazepam	1
Amphetamines	MDMA/MDA*	14
	MDMA	13
	Amphetamine	7
	Methamphetamine	6
	MDA	3
Cocaine	Cocaine/Benzoylecgonine*	34
	Cocaethylene	12
	Benzoylecgonine	7
	Cocaine	5
Opiates	Oxycodone	3
	Morphine	2
	6-Monoacetylmorphine	1
	Hydrocodone	1
NPS	Ethylone	56
	Methylone	24
	Alpha-PVP	12
	Dimethylone	7
	Butylone	4
	4-FA	2
THC	THC/THC-COOH*	152
Other Compounds	Dextromethorphan	1
	Dehydronorketamine/Norketamine/Ketamine†	1
	Citalopram†	1

*Positive sample contained both parent and metabolite.
†Oral fluid samples screened positive for dehydronorketamine, norketamine, and/or ketamine and citalopram, but these analytes are not within the scope of the confirmatory method.

Oral Fluid to Blood Ratios

In comparing the five positive methylone samples, there was a correlation (R^2=0.92); however, with a limited number of samples the correlation could easily be skewed. For the four paired alpha-PVP samples, there was a not a strong correlation (R^2=0.62). The oral fluid to blood ratios present some of the first reported ratios for NPS, however, with the limited number of paired samples definitive conclusions regarding oral fluid to blood ratios cannot not be determined. Uniformly, the NPS concentrations were higher in oral fluid than in the corresponding blood samples with oral fluid to blood ratios ranging from 2.78 to 22.48. For THC, the correlation between blood and oral fluid concentrations was poor (R^2=0.028), with oral fluid to blood ratios ranging from 0 to 279 (mean 25.5, median 1.9).

Metabolite Identification

Alpha-PVP

Alpha-PVP was seen to undergo extensive phase I metabolism, and eight phase I metabolites were identified in the *in vitro* assays with HLMs (Figure 3). Alpha-PVP metabolites produced using HLM were successfully identified in human blood, urine, oral fluid samples. The primary blood metabolites include one of the 5-OH-PVP diasteromers and the 2"-oxo-PVP metabolite. This represents the first report of detecting alpha-PVP metabolites in blood and oral fluid. Moreover, in two blood cases and three oral fluid cases screening only for the parent compound would have resulted in the sample being negative; however, the presence of the 5-OH-PVP would indicate prior use. Urine samples were found to contain additional metabolites with the most prevalent being the following: 5-OH -PVP, butylamino OH-Alkyl- PVP, 2"-oxo-PVP, and OH-alkyl-PVP.

Dimethylone

Dimethylone incubations were compared to results from *in vitro* metabolism of methylone, as dimethylone was seen to metabolize into methylone by N-dealkylation, and then further by demethylenation of methylone. Dimethylone also metabolized by demethylenation into 3,4-dihydroxy-N,N-dimethylcathinone followed by methylation to either 3-hydroxy-4-methoxy-N,N-dimethylcathinone or 4-hydroxy-3-methoxy-N,N-dimethylcathinone. The proposed metabolic pathway of dimethylone is shown in Figure 4. Dimethylone metabolites produced using HLM were successfully identified in human blood, urine, and oral fluid samples. The main metabolite in blood and oral fluid samples was methylated dimethylone and in urine was the hydroxylated dimethylone. Generally, when dimethylone is confirmed in a sample, methylone is also confirmed. The presence of methylone in a sample cannot be definitively identified as a product of metabolism or as the result of co-ingestion.

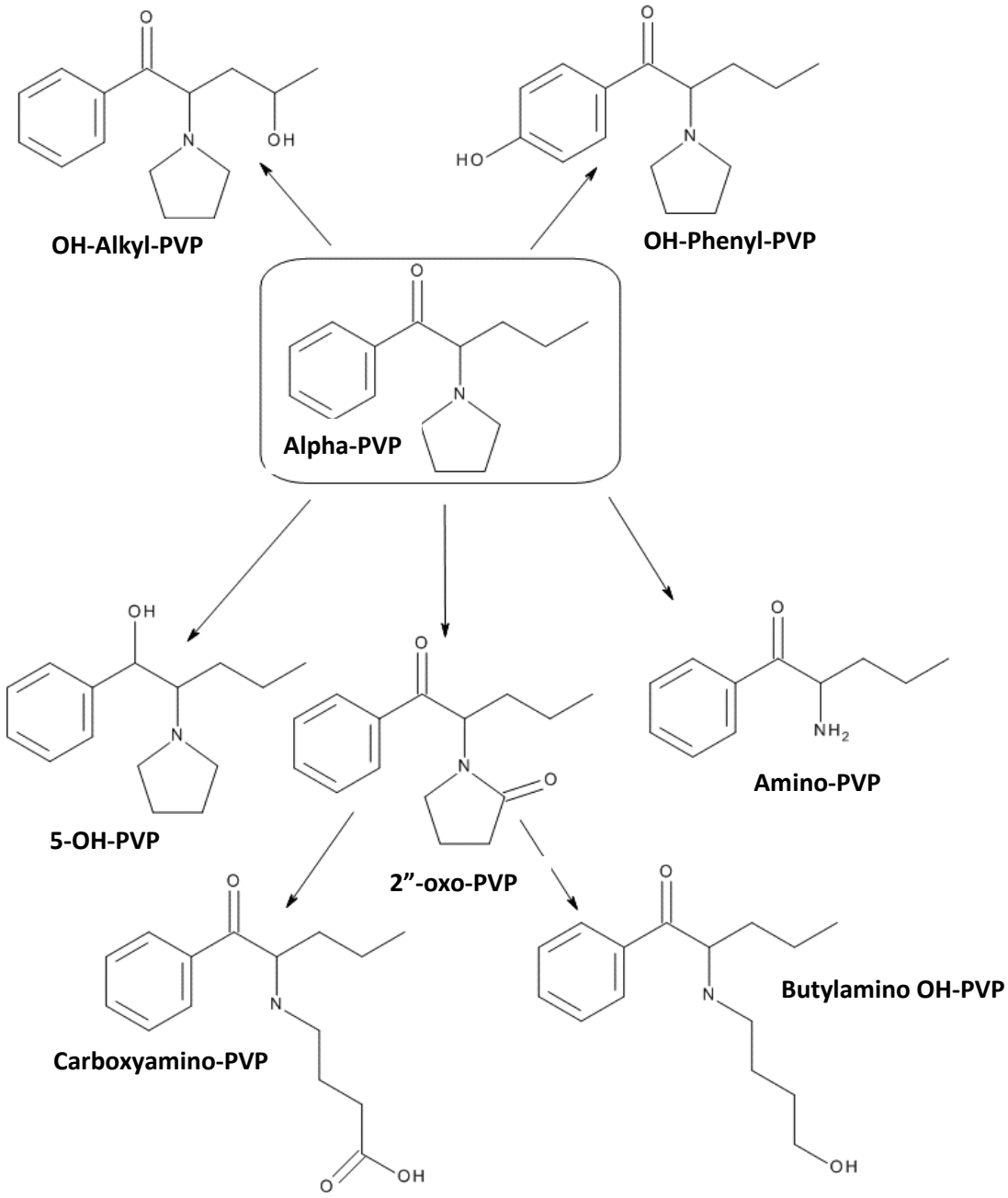

Figure 3 Proposed metabolic pathway of alpha-PVP as seen in HLM incubations.

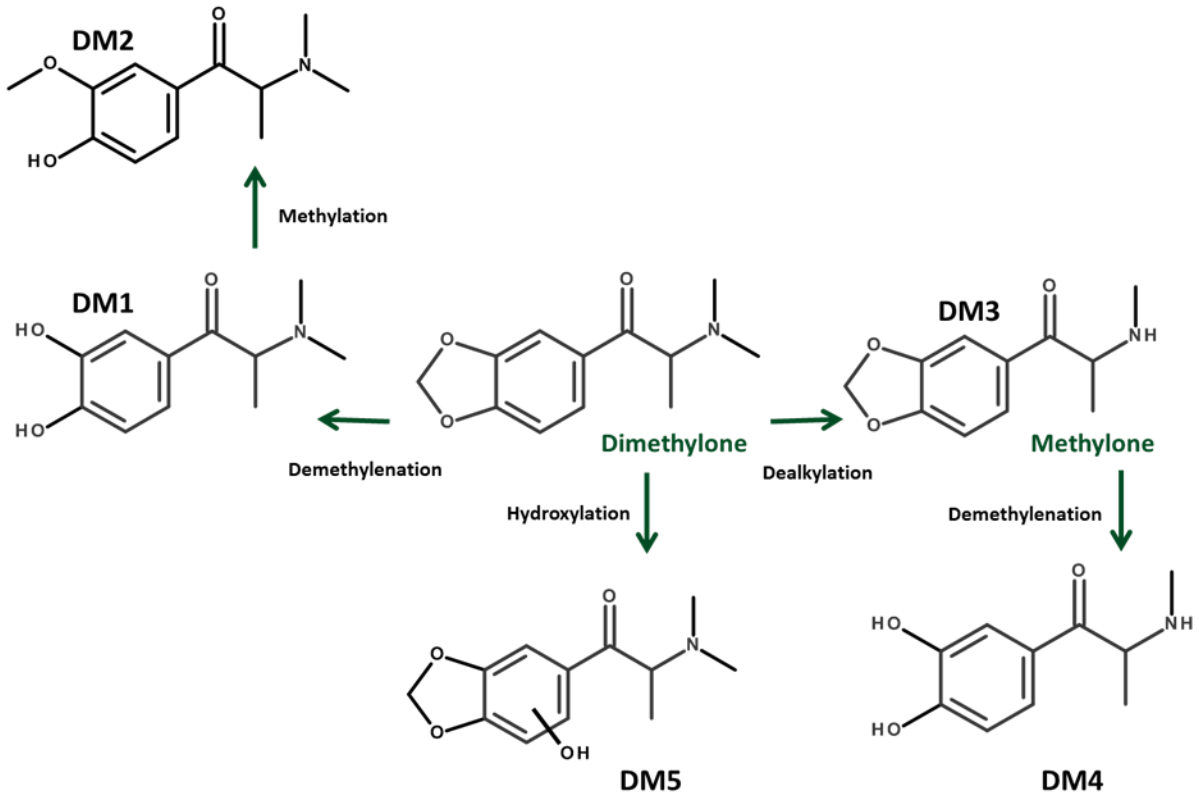

Figure 4 Proposed metabolic pathway of dimethylone as seen in HLM incubations.

CONCLUSIONS

The objectives of this project were to develop a better understanding of three aspects of the emerging designer drug phenomenon: 1) to study and verify the reported high incidence of use of drugs, particularly emerging NPS use among attendees at EDM scene; 2) to identify the compounds of concern and their metabolites in a trio of paired biological specimens – blood, oral fluid and urine, assessing in particular the value of oral fluid as an analytical matrix for detection of these drugs; 3) to identify novel metabolites for new or emerging drugs whose metabolic fate has not been completely studied in humans, but that were identified in the cohort we studied; and 4) develop and share analytical methods and mass spectral libraries for screening and confirmation methods on various analytical platforms that would assist the forensic science community in detecting use of these drugs in investigations of criminal activity, drug use and possession, impaired driving, drug facilitated sexual assault and other violent drug related crimes.

The project has met its goals in each of these areas.

1) **Confirming High Rates of Drug Use in the EDM community:**
We have used a novel methodology working with a cooperative cohort of recreational drug using subjects in a high risk group to obtain important epidemiological information, and valuable authentic paired biological samples to investigate human metabolism and the identity of the most prevalent biomarkers for evidence of use of these drugs. The results of

this study support previously reported high rates of drug use within this population, especially with respect to NPS. Using this model, we were successful in obtaining samples of oral fluid and/or blood and/or urine from 396 subjects, along with important demographic and drug use history information.

Self-reported drug information collected during the survey provided the unique opportunity to compare user reports of what the subjects thought they had ingested, to what was confirmed in their biological samples. Half the subjects who had reported using MDMA, "Molly" or Ecstasy had a biological specimen confirmed positive for an NPS, suggesting that this population is extremely vulnerable to being sold counterfeit substances which likely contain drugs different from what the user may have been expecting or have had experience or tolerance to.

Of the 396 subjects that participated in our study, 27% were confirmed positive for an NPS and/or MDMA in one or more biological specimens. Excluding the samples that were completely negative for drugs or alcohol (n=102), the positivity rate for an NPS and/or MDMA in drug users at the event increases to 36%. Within the population, 70 subjects (18%) were positive for NPS, excluding MDMA. With respect to the positive samples, the majority of those samples were confirmed for more than one drug, suggesting most of the drug-users within this population are poly-drug users who are at even greater risk for adverse events.

2) **Demonstrating the value of oral fluid versus blood or urine as an analytical matrix for detection of NPS and other drugs.**

Related to the biological specimens, there was good agreement between the findings in blood, urine and oral fluid with respect to the positive findings. The urine samples often contained more drugs, but this is an expected result as urine tends to retain drugs and metabolites and provide a longer detection window compared to blood and oral fluid. There was good agreement in assessing drug positivity in the subject pool between the results from blood and oral fluid samples. This provides support that oral fluid is a viable specimen for detecting recent drug use. In comparing the blood concentrations to oral fluid concentrations, specifically for NPS, the oral fluid samples had higher concentrations of drug present supporting the use of oral fluid as an alternative to blood given the easier collection process and limited ability to adulterate the samples.

3) **Identifying novel metabolites for new or emerging drugs whose metabolic fate has not been completely studied in humans.**

Through this project, we optimized a robust and reliable method for producing metabolites *in vitro* using HLMs, which were used to produce proposed metabolic pathways for alpha-PVP and dimethylone as well as create metabolite libraries with these proposed metabolites. For alpha-PVP, metabolites were identified in all three matrices and in some cases could be used to indicate prior ingestion in the absence of the parent drug. The primary metabolites included one of the 5-OH-PVP diasteromers and the 2"-oxo-PVP. With respect to dimethylone, the main metabolite in blood and oral fluid samples was

methylated dimethylone and in urine was the hydroxylated dimethylone. Generally, when dimethylone is confirmed in a sample, methylone is also confirmed, however, the presence of methylone in a sample cannot be definitively identified as a product of metabolism or as the result of co-ingestion.

4) **Developing and validating analytical methods and mass spectral libraries for screening and confirmation methods**

Several screening approaches ranging from immunoassay, RapidFire tandem mass spectrometry, gas chromatography/mass spectrometry and a broad-based screening approach using exact mass (LCTOF) have been evaluated. Due to the diversity and continual emergence of new compounds, broad-based screening using LCTOF provides the most comprehensive scope with the greatest chance of identifying emerging compounds. In the course of the project we developed and validated an LCTOF method for the screening for over 250 compounds, including approximately 80 NPS drugs and their metabolites. In addition, we developed a catalog of a mass spectral data that will be shared through our website and downloadable for use by the forensic science community.

Implications for policy and practice

Based on the findings from this project, we identified implications for each of the areas addressed in the conclusions section.

1) **Confirming High Rates of Drug Use in the EDM community:**

Our work confirmed NPS drug use within the EDM community and is an integral part of the EDM culture. The findings of this study reinforce the value of this sample collection approach in identifying market changes, especially the quick turnover with respect to the relative popularity of different NPS. At the event in 2014, the majority of samples collected were positive for methylone and alpha-PVP, however one year later, not a single case screened positive for alpha-PVP. The 2015 data suggest that the market had moved to ethylone, an isomer of methylone, but which is not currently specifically scheduled in the United States. The data support the influence of DEA scheduling actions on the NPS market, and suggest that more rapid scheduling actions could further pressure on the market to combat availability.

2) **Demonstrating the value of oral fluid versus blood or urine as an analytical matrix for detection of NPS and other drugs.**

The data from both years of the study showed that oral fluid is an ideal matrix for large scale sample collection from a cooperative survey population, and subjects were very willing to provide oral fluid samples given the easy, noninvasive collection procedure. In addition, the oral fluid analytical results demonstrated that the positivity rate for oral fluid testing for all recreational drugs and NPS was highly correlated with the blood test results, and reflected the degree of drug use in the subjects as well as blood. The parent drug concentrations in the oral fluid were typically higher than the blood concentrations,

although not quantitatively correlated. Based on this experience, we recommend that oral fluid can be used in place of blood to expedite collection from a larger sample population.

3) **Identifying novel metabolites for new or emerging drugs whose metabolic fate has not been completely studied in humans.**

The approach of collecting paired blood/oral fluid and urine samples, and comparing the results of the parent drugs and metabolites identified in each with the results of HLM incubations with the target drugs proved to be an effective approach for verifying the identity of the most significant markers for NPS drugs in biological samples. This will facilitate the choices of forensic laboratories in developing assays to detect illicit drug use in criminal investigations such as impaired driving and drug facilitated sexual assault. It will also assist in the identification of unknown metabolites in death investigation toxicology applications. Knowing the identity of the metabolites or markers also allows further work to be done investigating the toxicity of the drug, by being able to evaluate the metabolites for activity that may contribute to the main or side effect profile of the drugs.

4) **Developing and validating analytical methods and mass spectral libraries for screening and confirmation methods**

It is clear that immunoassay (EMIT, ELISA), which is currently used extensively for drug screening in forensic toxicology is not a realistic approach to the screening of NPS drugs. The structures are too varied to allow for significant cross reactivity, and the 12-18-month cycle time for raising antibodies developing and validating novel immunoassays would render the new tests out of date by the time they are available. Other alternatives need to be prioritized, such as high resolution mass spectrometric (HRMS) LCTOF, which is an invaluable tool that should be made more widely available to the forensic science community for the elucidation of the identities of unknown drugs.

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FINAL TECHNICAL REPORT (MAIN BODY)

Introduction

According to 2013 data from the National Survey on Drug Use and Health, an estimated 24.6 million Americans had used illicit substances within the prior month (1). The emergence of "designer drugs," "legal highs," or "club drugs," which refers to a broad category of products containing unregulated psychoactive constituents that are easily attainable via the internet, gas stations, and smoke or head shops, has created a growing trend in young adults as popular substances of abuse (2). Their widespread availability, ease of purchase, and not being included in routine drug testing are cited as part of their overall appeal and increased prevalence (3). In the United States, the rave culture, characterized by all-night dance parties and loud "techno-rock" and electronic dance music (EDM), has become a popular venue for these recreational drugs (4-5). Literature sources indicate that as many as 70% of attendees may be using recreational substances at these events (6). The use of novel psychoactive substances (NPS) at EDM festivals has been documented by surveys with EDM attendees and is reflected in discussion groups online associated with EDM culture (7-10). Within the last two years, several reports of fatal overdoses and non-fatal drug intoxications have stemmed from NPS use at EDM festivals within the United States.

Although the federal government and states are attempting to regulate these substances, NPS are still readily available to users because they are easily manufactured and manipulated to avoid legislation (11-12). In 2014, the European Monitoring Centre for Drugs and Drug Addiction (EMCDDA) reported an additional 101 NPS drugs found during seizures for the first time(13), which has steadily been increasing since 2005. According to the Global Drug Survey, the biggest users of research chemicals and legal highs were those in USA with over one in five of the greater than 1500 self-nominating respondents having used one of these compounds in the last year (14). According to the National Forensic Laboratory Information System (NFLIS), which collects drug identification results and associated information from drug cases submitted to and analyzed by Federal, State and local forensic laboratories, cases reported to contain synthetic cannabinoids and synthetic cathinones dramatically increased between 2010 and 2013 (15). For synthetic cannabinoids, reports increased from 469 in 2010 to 37,500 in 2014, and for synthetic cathinones the increase was from 142 in 2010 to 274,862in 2013 (15).

The creation of synthetic cannabinoids originated in the 1980-90s in various educational and research laboratories. These compounds, including JWH-018, JWH-250, JWH-122 and others, quickly became popular synthetic cannabinoid compounds added to herbal blends for smoking purposes. Synthetic cannabinoids include not only the JWH family of compounds, but also several other compounds designed with binding affinity for the CB1 and CB2 receptors. Based on their binding affinities to either the CB1 or CB2 receptors, adverse effects that are associated with cannabinomimetics include psychosis, seizures, anxiety, agitation, irritability, memory changes, sedation, confusion, tachycardia, cardiotoxicity, chest pain, nausea, vomiting, somnolence, dilated pupils, appetite changes, and tolerance (16). The drug user community

continues to synthesize new compounds to add to the botanical material and marks their products as “not for human consumption” and as “herbal incense” to skirt existing regulations.

Synthetic cathinones, including methylenedioxypyrovalerone (MDPV), mephedrone, and pentedrone, and more recently alpha-PVP have recently appeared in the drug user market as legal stimulants. Cathinones have been popularly referred to as “bath salts” or “plant food” (labels under which they are sold) and their effects are generally similar to those experienced after the use of cocaine, methamphetamine or methylenedioxymethamphetamine (MDMA) but often more exaggerated (17). Intense stimulation is often accompanied by negative effects such as tachycardia, hallucinations, paranoia, psychosis and erratic behavior (18). A similar related drug class of phenethylamine based compounds has also emerged as a source of recreational drugs. This class is primarily comprised of “2C” series compounds which are ring-substituted phenethylamines with similar structures to MDMA. Literature reports indicate that at least five deaths have been related to 2C intoxication, mostly attributed to excited delirium and paranoia (5). Additional noted undesirable effects include tachycardia, hyperthermia and seizure activity (19). However, limited epidemiological studies exist that are able to detect and compare these compounds in blood, urine and oral fluid as well as identify their metabolites (20). A further related class of N-benzylmethoxy substituted phenethylamines (NBOME’s) has also appeared (21).

Inevitably, published methodologies for the detection and identification of NPS lag behind the market itself. As the illicit drug market is in dynamic flux, the emergence of new compounds is continuous, creating challenges for both the criminal justice community as well as the forensic science community. There is no structured effort to identify novel compounds in toxicologically tested populations (such as workplace), and the identification of NPS in use is often serendipitous. This ad-hoc process has hindered the effectiveness of the forensic science community as the decision about which target compounds to prioritize for inclusion in the scope of analysis is based on out of date information. Emergency medical care, user education, law enforcement, distribution interdiction, drug treatment and intervention all depend on an understanding of which substances are prevalent, emerging, and residual in this user population. These limitations are further compounded by the lack of availability of analytical standards to use as drug reference material. Many of the material manufacturers, who synthesize reference materials for the forensic science community and research facilities among several other entities, lack the data supported information regarding what new drugs are being abused on the market and should be prioritized for preparation and sale.

Continuous monitoring of drug trends is necessary in order to make sure drug testing panels are targeting relevant compounds. Adverse events associated with these drugs are increasing significantly as reflected in emergency room, and medical examiner data sets (22-23). However, because of limitations associated with the lack of confirmatory testing procedures for the newest compounds and the inability of most forensic laboratories to offer updated and comprehensive testing, only a fraction of the problems associated with emerging NPS drugs are recognized and reported. The collection of paired blood, urine, and oral fluid specimens from authentic recreational users allows for the compilation of valuable information to be obtained

from a population at risk for using some of the most novel recreational chemicals on the market.

Oral fluid has become a popular biological matrix for forensic use based on its rapid collection and ability to provide results immediately, allowing for correlation of levels to pharmacodynamic effects (24). Recently, it has been subjected to several large-scale roadside studies, including the Roadside Testing Assessment I and II (ROSITA) and Driving Under the Influence of Drugs, Alcohol and Medicines (DRUID). These studies evaluated commercially available oral fluid screening devices by establishing roadside checkpoints where drivers were asked to voluntarily submit oral fluid samples that would be screened for drugs of abuse and selected therapeutic compounds (24-26). Although these valuable studies provided useful data concerning drug use of random driving populations, they did not evaluate a targeted group of high-prevalence drug users for novel recreational drug use. Additionally, most of the literature regarding the use of oral fluid has compared the use of on-site collection devices and the screening results they generate to compounds confirmed using either serum/plasma or urine (27-28). This data provides limited correlations between the concentrations in oral fluid compared to the concentrations found in the blood, which is used as the primary indicator of recent use and impairment. This study sought to compare oral fluid and blood samples to allow for the correlation of concentrations between compounds detected in these two specimens to be made, strengthening the assessment of oral fluid as a viable biological matrix for forensic use.

Further, metabolic studies for novel drugs remain limited, and generally metabolite elucidation occurs some later time after establishing the identity of the parent compound, if at all. Current research shows that many of the parent compounds are unstable or extensively metabolized, indicating the importance of determination of the identity of metabolites and breakdown products in order to detect use of these emerging drugs in urine samples (29). Because of the nature of synthetic drugs and the inability to perform ethical human trials with these potentially dangerous drugs, human dosing studies are not an option, making this opportunistic study of a drug using cohort an invaluable alternative. Much of the existing research on metabolism of emerging NPS is limited to *in vitro* and relies on pre-existing analytical data, however *in vitro* metabolic profiles, while of qualitative value, frequently do not reflect the relative prevalence of metabolites following ingestion, and consequently the most appropriate target compounds for which to develop lab tests (30). With respect to the identification of metabolites in authentic specimens, these investigations are limited to animal specimens or human urine. Very few reports have examined the extent of these metabolites in blood samples.

Alpha-pyrrolidinopentiophenone, also known as α-pyrrolidinovalerophenone, or simply abbreviated as alpha-PVP, is a stimulant which belongs to the pyrrolidinophenone family and is related to 4-methylenedioxypyrovalerone (MDPV) that appeared early on in the illicit drug markets (31). On the street, currently known as “Gravel” or “Flakka,” alpha-PVP is commonly distributed as tablets, capsules, or powders (31) and users report routes of administration including oral, sublingual, insufflation, and vaporization (32). User reports of effects include

stimulatory effects, euphoria, increased heart rate, decreased focus, and nausea, with hallucinations being associated only with very high doses (32-33). The mechanism of action producing these effects is inhibition of dopamine, serotonin, and norepinephrine transporters for reuptake (31, 34). After reports of intravenous abuse by multidrug users, this compound was scheduled as a controlled substance in March 2014 (35).

Alpha-PVP, though increasingly popular as a drug of abuse, remains relatively unstudied. Several publications exist concerning the metabolic products of this drug characterized in rat and human urine (31, 36-38). The metabolic pathways proposed include hydroxylation at both the alkyl chain and on the phenyl ring, oxidation of the pyrrolidine ring to a lactam and then to a carboxylic acid, reduction of the ketone and degradation of the pyrrolidine ring to a primary amine (31, 36-38). Human urinary metabolites identified include oxidation of the pyrrolidine ring to a lactam and the reduction of the ketone to an alcohol as well as degradation of the pyrrolidine ring to a primary amine, and the product of this degradation followed by reduction of the ketone to an alcohol (36-38).

Beta-keto-3,4-methylenedioxydimethylamphetamine (bk-MDDMA), more commonly referred to as dimethylone, is structurally similar to the more commonly abused methylone, differing only by a second methyl group on the amine. Methylone is the β-keto derivative of MDMA, and is one of the compounds often present in drugs distributed under the label “Molly”. In 2011, methylone was placed on the list of Schedule I substances by the DEA. As of the end of December 2015, dimethylone remains unscheduled directly, although its positional isomer butylone was temporarily scheduled as schedule I alongside alpha-PVP in 2014. Users report that dimethylone has effects very similar to methylone, but most report much weaker activity (33), while some user reports seem to suggest that dimethylone may be stronger in effect than its demethylated analogue (39). Effects reported include euphoric, empathogenic, and stimulatory effects after administration orally or by insufflation (33, 39).

Dimethylone remains largely unstudied, with little information available in either the scientific literature or within online drug communities. However, much work has been done concerning the metabolism of methylone, ethylone, and other closely related compounds (37-38, 40-43). In these compounds, the routes of metabolism include N-dealkylation (37, 40-43), N-hydroxylation (41, 43) β-keto reduction (37, 42-43), and demethylenation followed by O-methylation (40-43).

The rapid evolution of the NPS drug market has resulted in limited or delayed information regarding recreational drug use trends and appropriate markers for use in biological specimens. The collection of multiple paired blood, urine and oral fluid samples provided a comprehensive approach for analysis that included: identification of parent drug; the presence of metabolites and their relative prevalence in authentic specimens; ability to investigate the viability of oral fluid a as a noninvasive biological specimen for confirmation of drug ingestion; relative concentrations of NPS in blood compared to oral fluid, investigation of self-reported drug use relative to the prevalence of individual drugs in biological specimens and overall drug use trends for EDM festival attendees.

Methods

Human Subjects

All research was Institutional Review Board (IRB) reviewed, approved and conducted in full compliance with U.S. Federal Policy for the Protection of Human Subjects (Basic DHHS Policy for Protection of Human Research Subjects; 56 FR 28003). Arcadia University's Committee for the Protection of Research Subjects: Institutional Review Board (IRB) reviews all research involving human subjects, regardless of funding source, to ascertain that the rights and welfare of subjects are being protected. The IRB is responsible for assuring that recruitment efforts are not misleading or coercive to the research subject and that their participation is voluntary with the option to withdraw at any point in time. All projects using human subjects are reviewed no less than annually.

Sample Collection

Over the course of two years, a total of 396 (>18 y.o.) human volunteers (188 males; 127 females; 81 unidentified) were recruited for this study at an EDM festival in Miami in the spring of 2014 and 2015. Volunteers were verbally recruited by peer recruiters, who approached potential volunteers on their way to the main entrance of the event. The purpose and significance of the project were thoroughly explained to each volunteer. If preliminarily agreeable to participate, subjects were escorted a short distance to the study site. Prior to signing a statement of informed consent agreeing to participate in the research, participants were allowed the opportunity to ask questions related to the study and asked if they had taken any substances of abuse on that day. Any individual who answered "yes" to that question was excluded from the study. Additional exclusion criteria included individuals deemed unable to donate the required blood, urine and oral fluid specimens, individuals who appeared too visibly intoxicated to give consent or could not understand the study as described. As no health care associated information was collected, HIPPA authorization was not required. Participants were given a unique identification number that linked survey data and samples. Subject names were not collected or associated with samples in any way. Volunteers who donated any sample were given a bottle of water, and volunteers who provided all three specimens (blood, urine and oral fluid) were given at $20 gift card.

Survey Data: Volunteers were asked a series of pre-approved questions that included age, gender and whether or not they had taken any medication or recreational drug with the last week. Participants who answered "yes" to that question were asked a series of follow-up questions about what substances they had ingested, symptoms experienced while taking the substance, method of ingestion, dosage, and how long ago they had taken that substance (Appendix A).

Blood Samples: Donors were escorted into a private collection facility where two samples of whole blood (~7 milliliters each) were collected from the antecubital vein by the trained phlebotomist. The blood was drawn into a sterile vacuum tube containing an anticoagulant and a preservative. Samples were initially stored refrigerated (4˚C) prior to being frozen (-80˚C) until analysis.

Urine Samples: Donors were directed to deposit a urine sample into a sterile collection cup provided by the research team in a private lavatory. Samples were initially stored refrigerated (4˚C) prior to being frozen (-80˚C) until analysis.

Oral Fluid Samples: Oral fluid samples were collected using the Immunalysis Quantisal™ device. As per manufacturer's instructions, donors were directed to place the sorbent pad collector under the tongue and close their mouth until the adequacy indicator turned blue, indicating the approximately one milliliter (mL) of sample had been collected. The collector was then transferred into the transport tube, which contains three mL of stabilizing buffer. Samples were initially stored refrigerated (4˚C) prior to being frozen (-80˚C) until analysis.

After each sample collection, signed informed consent documents and survey data (unlinked) were transported back to Willow Grove, Pennsylvania for storage and tabulation. Blood and oral fluid samples were shipped overnight on dry ice to The Center (Willow Grove, PA) for analysis. Urine specimens were shipped overnight on dry ice to the Armed Forces Medical Examiner's Office: Division of Forensic Toxicology (Dover, DE).

Sample Analysis

Initially, all survey data, including demographic information, such as age and gender as well user reports of all medicinal and recreational drugs ingested within the last week, method of ingestion, and dosage were compiled into an Excel spreadsheet for comparison and management of the data. All biological samples were initially screened for common drugs of abuse, therapeutic compounds, and emerging NPS by the appropriate methods described below. Any sample that screened positive for one or more drugs were sent for confirmatory analysis and quantitation, when appropriate. An overview of the biological sample analysis including the location for the analysis and analytical platform for both screening and confirmatory methods is shown in Figure 1.

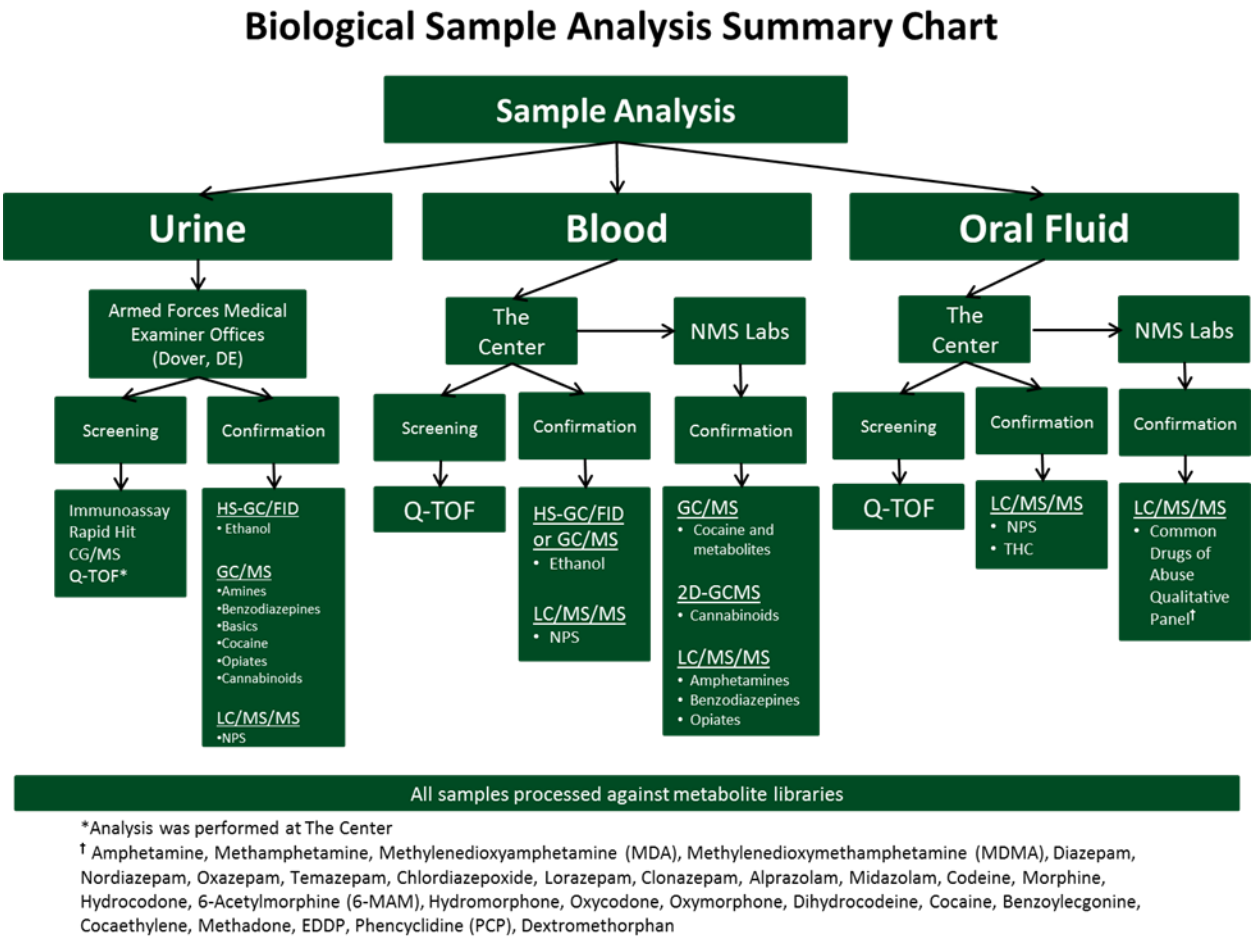


Figure 1 Summary of biological sample analysis by specimen type including location of analysis and analytical platform for screening and confirmatory methods.

Blood Samples: Upon arrival at The Center, one of the two blood samples collected at the event was thawed to allow the sample to be subdivided into 0.5 mL and 1 mL aliquots which were refrozen until needed to prevent the possible degradation of unstable compounds due to multiple freeze-thaw cycles**.**

Alcohol – Blood samples were analyzed for the presence of ethanol over a range from 10-400 mg/dL. Samples were prepared by diluting 100 µL of sample with 900 µL of 0.01% n-propanol using a Hamilton Microlab 600 Series Pipettor Dilutor. Samples collected in 2014 were analyzed using a Headspace Autosampler 7694 Hewlett Packard 5890N gas chromatograph flame ionization detector on a 30 meter Restex Rtx®-BAC PLUS 1 (320 µm diameter, 1.80 µm film thickness) column with an initial injection temperature of 240°C and an isothermal temperature program at 70 °C with a total the run time of 3.5 minutes. Samples collected in 2015 were analyzed using Combi Pal Headspace Autosampler Agilent 6890N gas chromatograph 5973N mass spectrometer on a 30 meter Zebron ZB-FFAP (0.25 mm diameter, 0.25 µm film thickness) column with an initial injection temperature of 250°C with a 50:1 spilt ratio and an initial oven temperature of 40°C ramping 20°C per minute to a final temperature of 150°C for a run time of

8.5 minutes. Quantitative values were determined by comparing samples to a calibration curve (10-400 mg/dL), which was required to have a correlation coefficient of 0.98 or greater as well as having controls at 50 mg/dL and 300 mg/dL quantitate within 10% of their target value.

Drug Screening – Prior to analyzing all blood samples, the screening method was developed and validated to be as comprehensive as possible for relevant therapeutic, abused and NPS substances to minimize the potential for false negatives. The method's performance was based on a validated method provided by the instrument manufacturer, and its performance was verified based on the validation guidelines as recommended by Scientific Working Group for Forensic Toxicology (SWGTOX), through a series of experiments outlined below. The goal of verification was to demonstrate the method was capable of successfully performing at the level of its intended use. A total of four different controls containing subsets of compounds in the analytical scope (totaling over 250 compounds), were tested in triplicate at various concentrations over the course of three days to evaluate the precision of the analytical method. The average ppm error, retention time, and response were calculated for each day as well as assessed over the course of the three days. The criterion for calling a sample positive included having mass error of less than 5 ppm, the retention time was required to be within a ±0.25 minute window of the retention time in library and the analyte was required to produce a response greater than 800.

Blood samples (0.5 mL) were made strongly basic using 0.1 M borax buffer (pH 10.4) and extracted into 70:30 n-butyl chloride/ethyl acetate. Samples were dried to completion at 33˚C and reconstituted in 200 µL 5 mM ammonium acetate (pH 3) and 0.1% formic acid in acetonitrile (90:10). All blood samples were screened using a Waters ACQUITY UPLC® I Class Waters Xevo® G2-S QTOF. Analytical separation was achieved using an ACQUITY UPLC® BEH C18 (2.1 mm x 150 mm, particle size 1.8 micron) column at 50˚C with a flow rate of 0.4 mL per minute and 5 µL injection. The Xevo® G2-S QTOF operated in positive electrospray ionization resolution mode (50-1000 m/z) with collision energy of 10-40 eV. The instrument was operated in resolution mode as a means to identify slight differences in mass measurements to assist in the identification of unknowns. Full method details can be found in Appendix B. Samples were processed against a library containing 1141 compounds. Criteria for calling a sample positive included: a clearly identifiable chromatograph peak within ±0.25 minutes of analyte in database, an observed mass of the molecular ion within ± 5ppm of mass in database, an observed mass of fragment ion within ± 2mDa, a response greater than 1500 (in the 3D data), and the presence of internal standards.

Confirmation – Samples that screened positive for an NPS (alpha-PVP, butylone, ethylone, dimethylone, methylone, and/or 4-fluroamphetamine) were confirmed at The Center using a method developed in-house and validated according to the SWGTOX guidelines. (Validation parameters and performance characteristics can be found in Appendix C). Samples that screened positive for selected therapeutic drugs and common drugs of abuse were confirmed by NMS Labs.

NPS Panel (*Alpha-PVP, Butylone, Ethylone, Dimethylone, Methylone, and/or 4-fluroamphetamine*) – 0.5 mL of sample was extracted using a liquid/liquid extraction. Samples were made basic using borax buffer and extracted into 70:30 N-butyl chloride:Ethyl Acetate. Samples were analyzed at The Center using a Waters Quattro Micro tandem mass spectrometer with a Waters Acquity Ultra Performance liquid chromatograph system. Full method details can be found in Appendix B.

Amphetamines Panel (*Amphetamine, Phentermine, Methamphetamine, Phenylpropanolamine, MDA, Ephedrine, MDEA, Methylephedrine, MDMA, Pseudoephedrine, Phendimetrazine, Norpseudoephedrine, Phenmetrazine, Selegiline*) – 200 µL of sample was combined with 200 µL of Trichloroacetic Acid (TCA) and centrifuged with 100 µL of the supernatant was transferred for analysis. Samples were analyzed at NMS Labs using Waters Micromass Quattro Premier tandem mass spectrometer coupled to a Waters Acquity Ultra Performance liquid chromatograph. Full method details can be found in Appendix B.

Benzodiazepines (*Alprazolam, Hydroxyalprazolam, Triazolam, Hydroxytriazolam, Midazolam, Estazolam, Lorazepam, Clobazam, Diazepam, Nordiazepam, Oxazepam, Temazepam, Clonazepam, 7-Aminoclonazepam, Flurazepam, Desalkylflurazepam, Hydroxyethylflurazepam, Chlordiazepoxide)* – 200 µL of sample was made basic using carbonate buffer and extracted into Methyl-t-Butyl-Ether (MTBE). Samples were analyzed at NMS Labs using Waters Micromass Quattro Premier or TQD tandem mass spectrometer coupled to a Waters Acquity Ultra Performance liquid chromatograph. Full method details can be found in Appendix B.

Cocaine and Metabolites (*Cocaine, Cocaethylene, Benzoylecgonine*) – 0.5 mL of sample was extracted using a UCT Clean Screen® 130 mg solid phase extraction column. Samples were buffered with phosphate buffer (pH 6) and eluted using Methylene Chloride:Isopropanol:Ammonium Hydroxide (78:20:2). Samples were analyzed at NMS Labs using an Agilent 6890 gas chromatograph 5973 mass spectrometer. Full method details can be found in Appendix B.

Opiates Panel (*Morphine, Hydromorphone, Oxymorphone, Codeine, Dihydrocodeine, Hydrocodone and Oxycodone*) – 200 µL of sample was extracted using a UCT Clean Screen® 130 mg solid phase extraction column and eluted with Ethyl Acetate:Ammonium Hydroxide:Isopropanol (78:2:20). Samples were hydrolyzed prior to extraction. Samples were analyzed at NMS Labs using a Waters TQS tandem mass spectrometer with a Waters Acquity Ultra Performance liquid chromatograph system. Full method details can be found in Appendix B.

Cannabinoids Panel (*Delta-9-THC, Cannabidiol, Delta-9-Carboxy THC, 11-Hydroxy-Delta-9-THC*) – 0.5 mL of sample was extracted using a liquid-liquid extraction. Samples were acidified using phosphoric acid in deionized water and extracted into Hexane:Ethyl Acetate:MTBE (80:10:10) and analyzed at NMS Labs using an Agilent 7890 gas

chromatograph 5975 mass spectrometer. Full method details can be found in Appendix B.

Urine Samples: Upon arrival at AFMES, the urine samples collected at the event were allowed to thaw at room temperature to allow for several 1 and 2 mL aliquots to be made for screening and confirmation purposes, which were then refrozen to prevent the possible degradation of unstable compounds due to multiple freeze thaw cycles**.** All analyses, processing and results were subject the criteria set forth by AFMES.

Alcohol – Urine samples were analyzed for the presence of ethanol over a range from 5-600 mg/dL. Samples were prepared by diluting 250 µL of sample with 2500 µL of working internal standard (2 mg/dL methyl-ethyl-ketone) and the caps were immediately crimped. Samples were analyzed using a 7679A Headspace Autosampler 7890N Gas Chromatograph Flame Ionization Detector on a 30 meter Restex Rtx®-BAC PLUS 2 (320 µm diameter, 0.60 µm film thickness) column with an initial injection temperature of 240°C and an isothermal temperature program at 45 °C with a total the run time of 5.0 minutes. Quantitative values were determined by comparing samples to a calibrator, as well as having controls at 50 mg/dL, 100 mg/dL, 200 mg/dL and 400 mg/dL quantitate within 10% of their target value.

Drug Screening – All urine specimens were screened at the AFMES Laboratory in Dover, DE, via several analytical techniques including immunoassay, gas chromatography mass spectrometry (GC-MS), liquid chromatography time of flight (LCTOF) and RapidFire tandem mass spectrometry (MS/MS).

Immunoassay Screen – Urine samples were screened via immunoassay analysis using a Hitachi Modular P Analyzer. The following reagents were utilized for presumptive testing: Roche Online DAT Amphetamines II and Opiates II, Microgenics DRI® Barbiturates, Ecstasy, Methadone, Oxycodone, Phencyclidine, and THC, Microgenics CEDIA® Benzodiazepine, heroin Metabolite (6-AM), K2 and LSD, and Siemens/Syva EMIT® II Plus Cocaine Metabolite. After calibration was performed, quality controls were analyzed. After every 20 specimens were analyzed, an additional set of quality controls were analyzed. The sample was prepared by aliquoting 0.5mL urine into the appropriate nested sample cup. The urine cutoff values are as follows: D-Methamphetamine (amphetamines) = 500 ng/mL, Secobarbital (barbiturates) = 200 ng/mL, Morphine (opiates) = 300 ng/mL, (-)-11-Nor-9-Carboxy-Delta-9-THC (THC-COOH) = 50 ng/mL, Benzoylecgonine (cocaine metabolite) = 150 ng/mL, PCP (phencyclidine) = 25 ng/mL, Nitrazepam (benzodiazepines) = 200 ng/mL, 6-acetylmorphine (6-AM) = 10 ng/mL, Oxycodone = 100 ng/mL, MDMA (ecstasy) = 500 ng/mL, LSD = 500 pg/mL, Methadone = 300 ng/mL, K2(JWH018-COOH) = 10 ng/mL.

GC/MS Base Screen – Urine samples (2 mL) were buffered by adding 3 mL 100 mM phosphate buffer (pH6.0). Next, 100 µL of internal standard mixture was added to the sample. Solid phase extraction was performed on each specimen by first conditioning the column (2 mL methanol followed by 3 mL DI H2O followed by 2 mL 100 mM

phosphate buffer (pH 6.0)), followed by loading the samples onto the UCT Clean Screen® DAU Extraction Columns, next a wash was performed (2 mL DI H2O, 2 mL 20% acetonitrile in DI H2O, 1 mL 100 mM acetic acid, dry column for 5 minutes, 2 mL hexane, 3 mL methanol, dry column for 10 minutes), with the final elution requiring 2 mL Elution Solvent (isopropanol, ammonium hydroxide, and methylene chloride). A volume of 10 µL of 10% HCl is added to each test tube prior to drying down the samples in a Zymark® Turbo Vap Evaporator. The samples are reconstituted with 50 uL of acetonitrile, vortexed, capped and centrifuged for 5 minutes prior to transfer in glass autosampler vial tubes with inserts. All urine samples were screened using an Agilent 6890 gas chromatograph coupled to an Agilent 5975 mass spectrometer. Analytical separation was achieved using a DB5MS column (20 m x 0.18 mm x 0.18 µm) with an initial temperature of 70°C (1 minute hold) followed by a ramp of 20°C/min with a final temperature of 300°C (5.5 minute hold) for a total run time of 17.5 minutes. Full method details can be found in Appendix B. Confirmation is achieved based on the retention time of the compound falling within ±2% of the retention time of the analyte in the standards and a full-scan mass spectrum of the compound matching a reference or library mass spectrum. The mass spectrum must have a minimum confidence of 70% compared to the reference library spectrum. If the compound identified by mass spectral analysis does not match one of the analytes in the standards, the suspected analyte must be run on the gas chromatograph under the same basic drugs screening conditions to verify a retention time match between the analyte standard and the compound in the specimen.

QTOF Base Screen – Urine samples (0.5 mL) were buffered by adding 3 mL 100 mM phosphate buffer (pH6.0). Next, 25 µL of internal standard mixture was added to the sample. Solid phase extraction was performed on each specimen by first conditioning the column (3 mL methanol followed by 3 mL DI H2O followed by 2 mL 100 mM phosphate buffer (pH 6.0)), followed by loading the samples onto the UCT Clean Screen® DAU Extraction Columns, next a wash was performed (3 mL DI H2O, 1 mL acetic acid, 3 mL methanol, dry column for 10 minutes), with the final elution requiring 2 mL Elution Solvent (isopropanol, ammonium hydroxide, and methylene chloride). A volume of 10 µL of 10% HCl is added to each test tube prior to drying down the samples in a Zymark® Turbo Vap Evaporator. The samples are reconstituted with 500 µL of mobile phase, vortexed, prior to transferring 100 µL into appropriately labeled LC autosampler vials with pre-slit caps. All urine samples were screened using a Waters Acquity I-Class UPLC with Xevo G2 QTOF. Analytical separation was achieved using a Waters Acquity HSS C18 150 mm x 2.1 mm x 1.8 µm column. Full method details can be found in Appendix B. Confirmation is achieved based on the retention time of the compound falling within ±2% of the retention time of the analyte in the standards or ±3% of the analyte in the Chromalynx database. The mass of the molecular ion for a suspected analyte must be within ±5ppm of the fragment mass listed in the Chromalynx database.

Rapid Fire tandem MS –All urine samples were screened for synthetic cannabinoids and synthetic cathinones using the Agilent RapidFire 365 coupled to an Agilent 6460 tandem

mass spectrometer. The automated high-capacity sample analysis is designed to provide a faster and more efficient analysis of samples in biological matrices by enabling direct, enzymatic detection of native analytes as well as analyzing multiple assays in a single run. Urine samples (50 µL) were added to individual wells of a 96-well plated and combined with 25 µL of internal standard and 25 µL of sodium hydroxide (0.3N) followed by 150 µL of diluent. The plate was sealed and centrifuged at 3000 rpm for five minutes. Samples are extracted using micro-scale inline solid phase extraction and transferred to the mass spectrometer. Full method details can be found in Appendix B.

Confirmation – Samples that screened positive for an NPS (alpha-PVP, butylone, ethylone, dimethylone, methylone, 2C-B and/or 4-fluoroamphetamine) or for selected therapeutic drugs and common drugs of abuse were confirmed by AFMES.

NPS Panel (*methylone, ethylone, butylone, 4-fluoroamphetamine, alpha-PVP, dimethylone, 2C-B*) – 2 mL of sample was extracted using a liquid-liquid extraction. Samples were made basic using sodium borate and concentrated ammonium hydroxide and extracted into chlorobutane. Samples were analyzed using an Agilent 1100 series liquid chromatograph coupled to a Sciex 3200 QTrap tandem mass spectrometer. Full method details can be found in Appendix B.

Amines Panel (*Amphetamine, Methamphetamine, Phenylpropanolamine, MDA, Ephedrine, MDMA, Pseudoephedrine*) –2 mL of sample was combined with 4 drops of KOH and underwent a liquid-liquid extraction. After reconstitution with ethyl acetate, samples were analyzed on an Agilent 6890 gas chromatograph coupled with an Agilent 5975 mass spectrometer. Full method details can be found in Appendix B.

Benzodiazepines (*Alprazolam, Hydroxyalprazolam, Hydroxytriazolam, Midazolam, Hydroxymidazolam, Lorazepam, Diazepam, Nordiazepam, Oxazepam, Temazepam, Clonazepam, 7-Aminoclonazepam, Desalkylflurazepam)* – 1 mL of sample was made acidic using 0.1M sodium acetate buffer before undergoing solid phase extraction using a Phenomenex Strata-XC column. After derivatization with 4:1 Acetonitrile:MTBSTFA w/1% TBDMCS, samples were analyzed on an Agilent 6890 gas chromatograph coupled with an Agilent 5975 mass spectrometer. Full method details can be found in Appendix B.

Cocaine and Metabolites (*Cocaine, Cocaethylene, Benzoylecgonine*) – 1 mL of sample was extracted using a UCT Clean Screen® 130 mg solid phase extraction column. Samples were buffered with phosphate buffer (pH 6) and eluted using dichloromethane:Isopropanol:ammonium hydroxide (78:20:2). After reconstitution with ethyl acetate, samples were analyzed on an Agilent 6890 gas chromatograph coupled with an Agilent 5975 mass spectrometer. Full method details can be found in Appendix B.

Opiates Panel (*Morphine, Hydromorphone, Oxymorphone, Codeine, Hydrocodone and Oxycodone*) – 2 mL of sample was extracted using a UCT Clean Screen® 130 mg solid phase extraction column and eluted with 2% ammonium hydroxide in ethyl acetate:methanol (2:1). Samples were hydrolyzed prior to extraction. After reconstitution with acetonitrile and BSTFA with 1% TMCS, samples were analyzed on an Agilent 6890 gas chromatograph coupled with an Agilent 5975 mass spectrometer. Full method details can be found in Appendix B.

Cannabinoids Panel (*Delta-9-Carboxy THC*) – 2 mL of sample was extracted using a liquid liquid extraction. Samples were first basified using KOH in deionized water and extracted with Hexane:Ethyl acetate (7:1). They were then acidified using hydrochloric acid and extracted into Hexane:Ethyl acetate (7:1) and were analyzed on an Agilent 6890 gas chromatograph coupled with an Agilent 5975 mass spectrometer. Full method details can be found in Appendix B.

Basics Panel - 2 mL of sample was extracted using a liquid-liquid extraction. Samples were first basified using ammonium hydroxide and sodium borate and extracted with chlorobutane. They were then back extracted by acidifying using sulfuric acid and extracted into chlorobutane for sample clean up. The samples were then made basic with potassium hydroxide and extracted into an chlorobutane. The samples were analyzed on an Agilent 6890 gas chromatograph coupled with an Agilent 5975 mass spectrometer. Full method details can be found in Appendix B.

Oral Fluid Samples: Upon arrival at The Center, the oral fluid sample collected with the Immunalysis Quantisal™ was thawed. The sorbent pad used to collect the sample was removed from the plastic applicator and transferred to a Sarstedt Salivette® and centrifuged at 4200 rpm to collect any residual saliva remaining on the sorbent pad. The remainder was added back to the Quantisal™ collection tube without the pad, and subsequently aliquoted into 0.5 mL sub-aliquots for refreezing to prevent the possible degradation of unstable compounds due to multiple freeze thaw cycles**.**

Drug Screening – In 2014, an additional oral fluid sample was collected onsite and screened using the Alere® DDS2 Mobile Test System (DDS2). The DDS2 is a hand-held oral fluid testing device that operates using lateral flow immunoassay technology and produces an electronic printout of the results. The device screens for six different analytes. The target analyte and cutoff are shown in Table 1. The results of the screen were compared to the confirmatory testing results generated with the Quantisal™.

Table 1. Scope and cutoff concentrations for the Alere DDS2.

Class	Target Analyte	DDS2 Cutoffs (ng/mL)
Amines	Amphetamine	50
	Methamphetamine	50
Benzodiazepines	Temazepam	20

Cannabis	THC	25
Cocaine	Benzoylecgonine	30
Opiates	Morphine	40

For purposes of this evaluation, since the field test results were specific to class of compounds amphetamines, benzodiazepines, opiates, cocaine and metabolites, methadone and cannabinoids), and the laboratory confirmations in oral fluid were specific to compound, comparisons were made by assigning any laboratory based positive to the corresponding drug class and comparing results by drug class. The data were assessed through the use of Receiver Operator Characteristic (ROC) analysis to determine sensitivity, specificity, accuracy, and percent positivity.

All oral fluid samples collected with the Quantisal™ were screened in the laboratory using a Waters ACQUITY UPLC® I Class Waters Xevo® G2-S QTOF. Prior to analyzing all oral fluid samples, the screening method was developed and qualitatively validated for 16 compounds (2C-B, 4-FA, alpha-PVP, benzoylecgonine, cocaethylene, cocaine, dimethylone, DOM, ketamine, MDA, MDMA, methamphetamine, methylone, norketamine, o-desmethyltramadol, and tramadol). These analytes were prepared at low, mid and high concentrations (Appendix C) and run in triplicate over three days. Parameters for evaluation included within and between run precision of the ppm mass error and retention time as well as the average response of the runs. The criterion for calling a sample positive included having mass error of less than 5 ppm, the retention time was required to be within a ±0.25 minute window of the retention time in library and the analyte was required to produce a response greater than 800. The stability of the target analytes at the low and high controls were assessed at -80^{o}C on days 7, 14, and 30 following the initial day of preparation. Processed sample stability of the low and high controls were evaluated at 24 hours, 48 hours, and 72 hours after the initial injection and monitored for a decrease in response relative to the initial injection. A decrease in response of greater than 20% indicated instability.

Oral fluid samples (0.5 mL) were made strongly basic using 0.1 M borax buffer (pH 10.4) and extracted into 70:30 n-butyl chloride:ethyl acetate. Samples were dried to completion at 33˚C and reconstituted in 200 µL 5 mM ammonium acetate (pH 3) and 0.1% formic acid in acetonitrile (90:10). Analytical separation was achieved using an ACQUITY UPLC® BEH C18 (2.1 mm x 150 mm, particle size 1.8 micron) column at 50˚C with a flow rate of 0.4 mL per minute and 5 µL injection. The Xevo® G2-S QTOF operated in positive electrospray ionization resolution mode (50-1000 m/z) with collision energy of 10-40 eV. The instrument was operated in resolution mode as a means to identify slight differences in mass measurements to assist in the identification of unknowns. Full method details can be found in Appendix B. Samples were processed against a library containing 1141 compounds. Criteria for calling a sample positive included: a clearly identifiable chromatographic peak within ±0.25 minutes of the analyte retention time in the database, an observed mass of the molecular ion within ± 5ppm of mass in database, an observed mass of fragment ion within ± 2mDa, a response greater than 1500 (in the 3D data), and the presence of internal standards.

Confirmation – Samples that screened positive for an NPS (alpha-PVP, butylone, ethylone, dimethylone, methylone, and/or 4-fluroamphetamine) or THC were confirmed at The Center using a method developed in-house and validated according to SWGTOX guidelines. (Validation parameters and performance characteristics can be found in Appendix C). Samples that screened positive for selected therapeutic drugs and common drugs of abuse were confirmed by NMS Labs.

NPS Panel (*Alpha-PVP, Butylone, Ethylone, Dimethylone, Methylone, and/or 4-fluroamphetamine*) – 1 mL of sample was extracted using a liquid/liquid extraction. Samples were made basic using borax buffer and extracted into 70:30 N-butyl chloride:Ethyl Acetate. Samples were analyzed at The Center using a Waters Quattro Micro tandem mass spectrometer with a Waters Acquity Ultra Performance liquid chromatograph system. Full method details can be found in Appendix B.

Oral Fluid Qualitative Confirmation Panel (*Amphetamine, Methamphetamine, Methylenedioxyamphetamine (MDA), Methylenedioxymethamphetamine (MDMA), Diazepam, Nordiazepam, Oxazepam, Temazepam, Chlordiazepoxide, Lorazepam, Clonazepam, Alprazolam, Midazolam, Codeine, Morphine, Hydrocodone, 6-Acetylmorphine (6-MAM), Hydromorphone, Oxycodone, Oxymorphone, Dihydrocodeine, Cocaine, Benzoylecgonine, Cocaethylene, Methadone, EDDP, Phencyclidine (PCP), Dextromethorphan*) – 0.5 mL of sample was extracted using a solid phase Strata-X-C column. Samples were eluted with 5% ammonium hydroxide in methanol into a test tube containing 50 µL of methanolic HCl (1%). Samples were analyzed at NMS Labs on a Waters TQD tandem mass spectrometer with a Waters Acquity Ultra Performance liquid chromatograph system. Full method details can be found in Appendix B.

THC (*THC*) – 0.5 mL of sample was extracted using a liquid-liquid extraction. Samples were acidified using phosphoric acid in deionized water and extracted into hexane:ethyl acetate:MTBE (80:10:10). Analysis was performed using an Agilent 1100 series liquid chromatograph coupled to an Agilent 6430 tandem mass spectrometer (LC-MS/MS). Full method details can be found in Appendix B.

Synthetic Cannabinoids (*JWH-018, AM-2201, JWH-081, JWH-122, JWH-210, UR-144, XLR-11, AB-FUBINACA, ADBICA, 5F-ADBICA, ADB-PINACA, ADB-FUBINACA, 5F-ADB-PINACA, JWH-018 Adamantyl, 5F-JWH-018 Adamantyl Carboxamide, JWH-018 Adamantyl Carboxamide, PB-22, AKB-48, 5F-PB-22, 5F-AKB-48, BB-22, AM-2201 Benzimidazole, THJ-2201, THJ-018, 5F-AB-001, AB-PINACA, AB-CHMINACA*) – 0.5 mL of sample was extracted using a solid phase Oasis HLB 60mg column. Samples were eluted with 2.0 mL of acetonitrile and analyzed by NMS Labs on a Waters Premier or TQD tandem mass spectrometer with a Waters Acquity Ultra Performance liquid chromatograph system. Full method details can be found in Appendix B.

Oral Fluid to Blood Ratios – Oral fluid to blood ratios were calculated for NPS for samples in which there were paired specimens, to assess the utility of oral fluid as a matrix for NPS

monitoring and investigate any quantitative relationship between the concentrations. The reported calculated concentrations for both blood and oral fluid samples were plotted and evaluated for linearity and correlation (r^2).

Metabolite Identification: *In vitro* metabolism studies using human liver microsomes (HLM) were performed for alpha-PVP, methylone, and dimethylone. An optimized method adopted from Tiller et. al. was used for all HLM incubations (40).

Each reaction mixture contained 5000 ng of substrate (drug), 50 µL of 10 mM NADPH solution, 25 µL of pooled HLMs (20 mg/mL), and 520 µL of phosphate buffer (100 mM, pH=7.4, with 10 mM MgCl2), to a final volume of 600 µL. Each drug was metabolized in duplicate on several days, alongside two diazepam incubations, which were included to ensure that the microsomes were still metabolizing effectively and as expected. Also included in the analyses were controls containing diazepam and no NADPH, controls containing the NPS and no NADPH, controls containing the NPS and no NADPH or microsomes, blanks run between each sample, and standard solutions of the drugs of interest (one containing diazepam, nordiazepam, and temazepam, and a second containing the NPS of interest).

Samples were incubated for two hours in a water bath at 37˚C. The reactions were stopped with 500 µL of acetonitrile. Each mixture was vortexed, centrifuged, and the supernatant was partially dried down and then filtered before transfer to autosampler vials. Samples were subsequently processed on a Waters ACQUITY UPLC® I Class coupled with a Waters Xevo® G2-S QTOF Analysis occurred by UPLC/Q-TOF using the Forensic Toxicology Screening Solution with UNIFI™. Analytical separation was achieved using an ACQUITY UPLC® BEH C18 (2.1 mm x 150 mm, particle size 1.8 micron) column at 50˚C with a flow rate of 0.4 mL per minute and 5 µL injection. The Xevo® G2-S QTOF operated in positive electrospray ionization resolution mode (50-1000 m/z) with collision energy of 10-40 eV. The instrument was operated in resolution mode as a means to identify slight differences in mass measurements to assist in the identification of unknowns. Full method details can be found in Appendix B.

The controls, standards, blanks, and diazepam were first evaluated for each run. Once it was verified that the method was producing metabolites, any peaks that were artifacts of the microsomes, via evaluation of the appropriate controls, were identified as such. The reaction mixtures from the parent drugs of interest were analyzed using the Binary Comparison tool in UNIFI™(Waters®), where a control incubation was compared to a reaction mixture and evaluated for any peaks of interest only present in the reaction mixture as well as by extracting the masses of suspected metabolites or commonly encountered metabolites. For each of these peaks of interest, the elemental composition tool was used to determine the molecular formula of the unknown metabolite and fragments of that metabolite. Potential metabolites' molecular ion masses and retention times were used to set up an MSMS run to obtain the fragments of these possible metabolites at varying collision energies. For the MSMS analysis, the incubations were performed with the previously described method, with the analysis including the drug(s) of interest and appropriate controls. The produced fragmentation pattern at each peak was used to suggest and confirm the structures of each proposed metabolite. A metabolite library,

which included proposed metabolites and supporting fragments, was created for alpha-PVP as well as one for methylone and dimethylone. All blood, urine, and oral fluid samples were processed against each metabolite library to evaluate the extent to which phase I metabolites produced *in vitro* could be found in authentic human specimens.

Unknown Analytes: All specimens collected were analyzed via a battery of analytical methods in order to fully investigate the compounds present as well as the anticipation of detecting unknown analytes.

RESULTS AND DISCUSSION

Survey Data: Survey data was obtained from 342 subjects. A total of 188 males (55%) and 127 females (45%) provided survey information, with 27 subjects not indicating a gender. The average age of the participants in was 22.5 years old (±5 years). In both 2014 and 2015, 72% of the respondents had reported using a medicinal substance or recreational drug within the past week (Note: In 2014, one person did not answer that question). The most common substance participants indicated that they had taken was marijuana, followed by alcohol and "Molly". "Molly" is a slang term, which previously has referred to MDMA, however, today the term is most commonly associated with methylone. Shown in Figure 2 are the percentages of responses for the most commonly reported recreational drugs used with the last week.

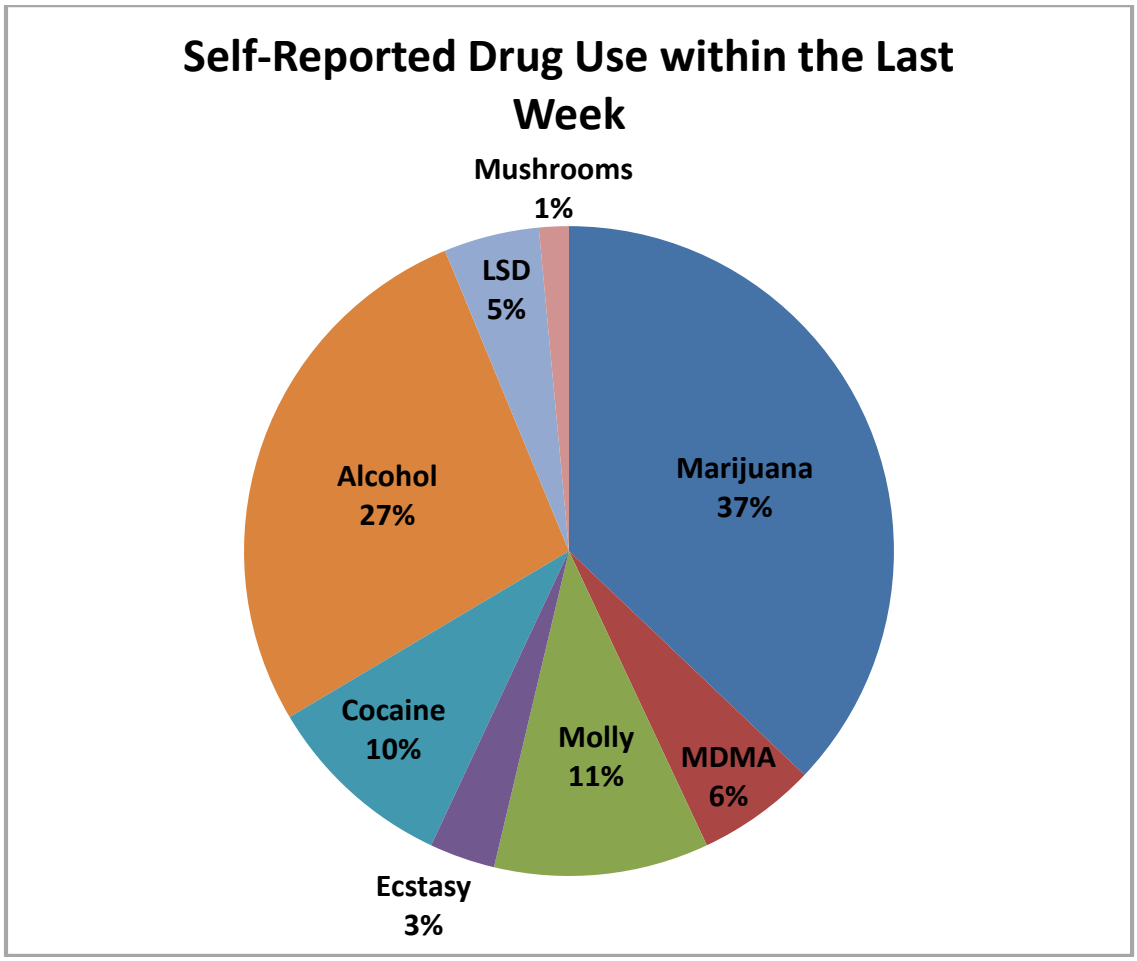


Figure 2 Festival attendees self-reported medicinal or recreational drug use within the last week among users reporting recent drug use.

In terms of NPS, 15% (n=60) of the subjects had reported using "Molly", MDMA and/or Ecstasy. A total of 28 samples (47%) were positive for an NPS, 9 samples were positive for MDMA (15%), 10 samples were positive for an NPS and MDMA (17%), and 13 of those samples were negative (22%). The most common NPS found in these 60 samples were ethylone (n=21, 35%), methylone (n=12, 20%), and alpha-PVP (n=8, 13%). There was a large discrepancy between what users reported taking and what was confirmed in their biological samples. Further, for users who had reported taking "Molly," there was a large amount of variability in terms of what was confirmed in the biological samples. Most of the biological samples confirmed positive for an NPS in users who had reported taking "Molly," meaning that users are often unknowingly ingesting highly potent synthetic compounds.

Users reported ingesting NPS such as "Molly", MDMA and/or Ecstasy orally either in a pill or capsule form, with most individuals taking between one to three capsules or pills. The average reported dose for MDMA was between 100 – 500 milligrams, while users reported taking between 100 milligrams up to 3 grams of "Molly".

Blood Samples

Alcohol – All blood samples (n=126) were screened for the presence of alcohol. Fifty samples were positive for ethanol (approximately 40% of the population) and the remaining 76 were negative. The average blood alcohol concentration was 102 mg/dL (±66) with a range of 10-304 mg/dL. With respect to the alcohol results, 66% of the samples (n=33) were positive for alcohol only, four samples (8%) were positive for alcohol and more than one drug, and 22% (n=11) were positive for alcohol plus one drug. (Note: Two samples were not included in the totals because they contained ethanol in addition to dimethylone and methylone, and it could not be determined if the drugs were co-ingested or if dimethylone had been ingested and metabolized to methylone). For the samples that were positive for alcohol plus a single drug, the drug results are as follows: THC (n=5), cocaine (n=4), tramadol (n=1), and alprazolam (n=1).

Drug Screening – Full verification data for the liquid chromatograph time-of-flight mass spectrometer can be found in Appendix C. The within run and between run ppm error and retention time error as well as average reported response of all runs were tabulated. The ppm error had to be <5 relative to the reported accurate mass in the library and retention time had to be within ±0.25 minutes of the reported library retention time. Samples that had a retention time outside of the ±0.25 minute window were subsequently updated in the library to the appropriate retention time, which was determined by running an analytical standard. Based on this verification, we discovered the system has an issue with primary amines. These small molecules generally fragment in the source causing the system to miss the parent mass and incorrectly identify false negatives. To address this issue, the library was updated with a unique fragment that was used as the parent mass. With these adjustments, the system preformed as intended and provided a reliable and robust screening method.

One hundred and twenty-six blood samples were screened over both years. The highest percentage of screen positive results was for NPS (28%), which was followed by cocaine (22%). Drugs listed in the "other" category included the following: diphenhydramine, sildenafil, aripiprazole, azithromycin, desloratidine, chlorpheniramine, acetaminophen, bupropion, dextromethorphan, cyclobenzaprine, fluoxetine, methylphenidate, and quetiapine. The screen positive results are showing in Figure 3.

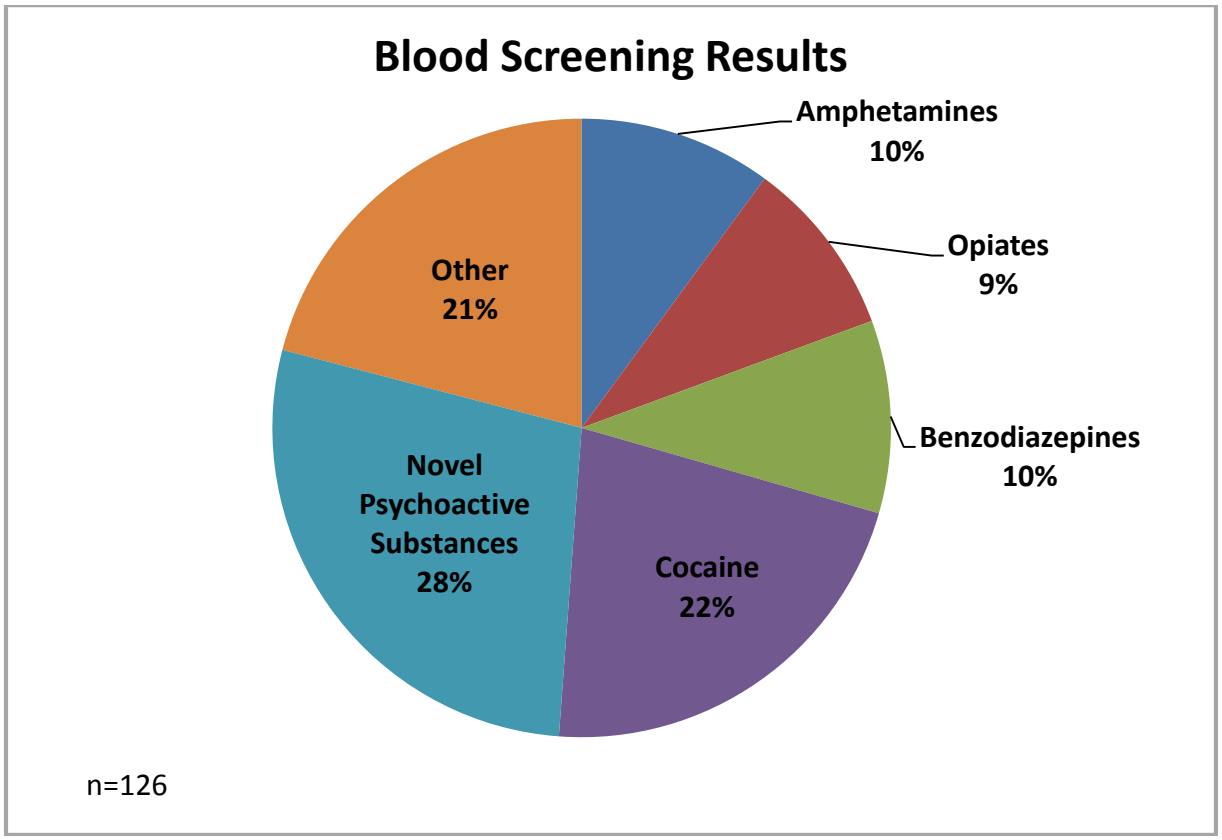


Figure 3 Blood screening results by drug class as determined by liquid chromatography quadrupole time-of-flight mass spectrometry.

With respect to NPS, the most common screen positive result was for alpha-PVP, followed by methylone, and subsequently ethylone/butylone/dimethylone. The last three compounds, ethylone, butylone, and dimethylone are all structural isomers with the same exact mass and not chromatographically resolved in the drug screen, requiring confirmatory testing to differentiate between them. It should be noted that all 10 of the alpha-PVP screen positive results were obtained in the samples collected in 2014. Shown in Figure 4 are the screen positive results specifically for the drugs within the NPS category over both years.

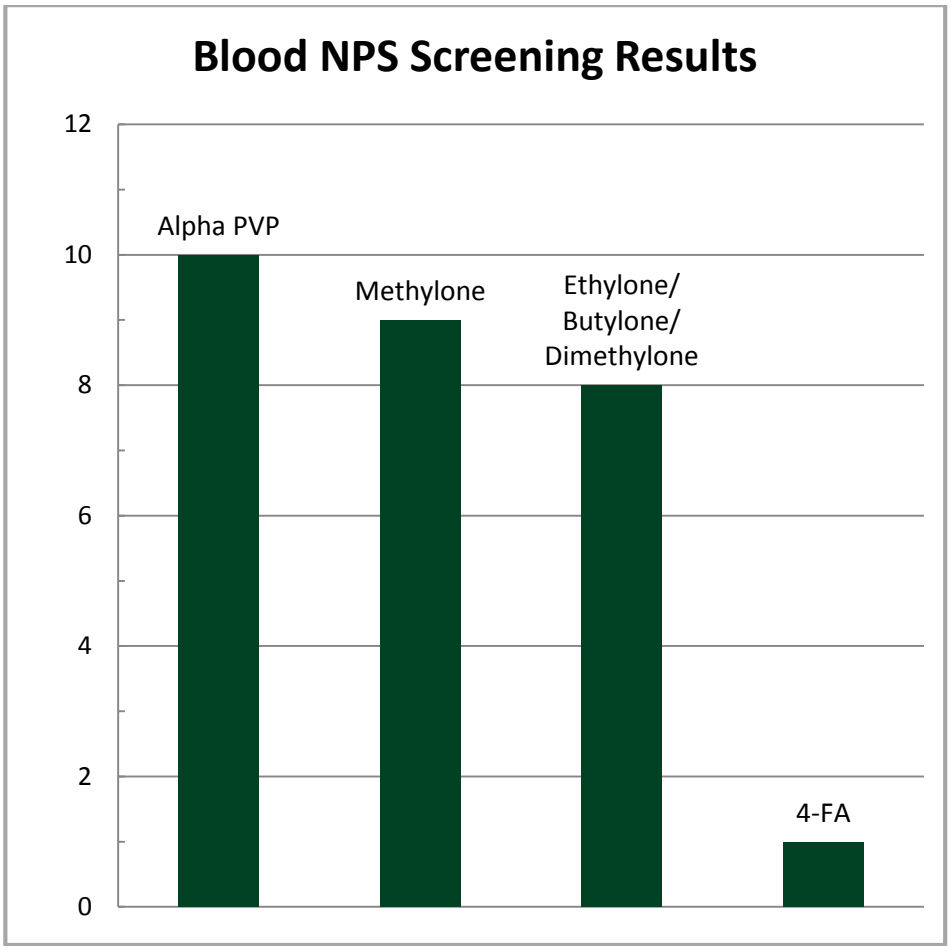


Figure 4 Number of blood sample that screened positive for an NPS compound.

Confirmation – The confirmatory results for each subject are listed in detail in Appendix D.

NPS Panel – Of the 18 samples that were sent for confirmatory testing, 14 of the samples (78%) were confirmed to have at least one NPS present in blood. Methylone was the most commonly confirmed NPS, which was detected in 9 cases, followed by dimethylone (n=6) and alpha-PVP (n=6). Results of all the confirmed NPS in blood are shown in Table 2.

Table 2 Confirmatory testing results for NPS detected in blood.

Analyte	Number of Positive Samples	Average Concentration (ng/mL)	Standard Deviation	Median (ng/mL)	Range (ng/mL)
Methylone	9*	89	±138	28	7-375
Alpha-PVP	6*	46	±43	45	8-87
Dimethylone	6*	611	±55	53	10-157
Ethylone	2	211	N/A	211	210-212
4-FA	2*	71	N/A	N/A	N/A
Butylone	1*	N/A	N/A	N/A	N/A

* Positive number of samples includes samples that had a result less than the limit of quantitation, but were determined to be positive based on a chromatographic peak at correct retention time and passing ion ratios (4-FA n=1, alpha-PVP n=2, butylone n=1, dimethylone n=1, and methylone n=2).

Amphetamines – A total of ten samples were sent for confirmatory testing with all ten samples confirming positive for one or more analytes within the scope of the method. The amphetamine panel blood results are shown in Table 3. Six blood samples were confirmed positive for MDMA, however, one sample was confirmed at a level below the limit quantitation and therefore could not be included in the average concentration, standard deviation or range results.

Table 3 Confirmatory testing results for amphetamines detected in blood.

Analyte	Number of Positive Samples	Average Concentration (ng/mL)	Standard Deviation	Median (ng/mL)	Range (ng/mL)
MDMA	6†	83	±106	57	7.5-270
Amphetamine	3	105	±85	150	6-160
MDA	3	13	±10	7.5	7-26
Methamphetamine	1	570	N/A	N/A	N/A

†One sample concentration less than limit of quantitation (5 ng/mL): result not included in average concentration, standard deviation, or range.

Benzodiazepines – With respect to benzodiazepines, 13 samples screened positive for the presence of one or more benzodiazepines. Of those 13 samples, 6 were confirmed positive for alprazolam (46% of the samples). One sample was positive for clonazepam and its metabolite 7-amino clonazepam. The confirmatory results for benzodiazepines are show in Table 4.

Table 4 Confirmatory testing results for benzodiazepines detected in blood.

Analyte	Number of Positive Samples	Average Concentration (ng/mL)	Standard Deviation	Median (ng/mL)	Range (ng/mL)
Alprazolam	6	30	±21	25	8.3-57
Clonazepam	1	41	N/A	N/A	N/A
7-amino clonazepam	1	40	N/A	N/A	N/A

Cocaine and Metabolites – Fifteen of the 16 (94%) samples that were sent for confirmatory testing on the cocaine and metabolites method were confirmed positive for cocaine and/or benzoylecgonine, which is the primary metabolite of cocaine. For one sample that contained benzoylecgonine, the sample was determined to contain the drug at a level below the limit (50 ng/mL) and could not be included in the average concentration, standard deviation or range determinations. Two samples were also confirmed for the presence of cocaethylene, which is formed when cocaine is co-ingested with alcohol. The results for this confirmatory method are shown in Table 5.

Table 5 Confirmatory testing results for cocaine and metabolites detected in blood.

Analyte	Number of Positive Samples	Average Concentration (ng/mL)	Standard Deviation	Median (ng/mL)	Range (ng/mL)
Benzoylecgonine	12†	275	±168	280	56-645
Cocaine	9	55	±41	45	21-130
Cocaethylene	2	34	N/A	34.5	27-42

†One sample concentration less than limit of quantitation (50 ng/mL): result not included in average concentration, standard deviation, or range.

Opiates – A total of nine samples were sent for confirmatory testing for opiates. The results of that testing are shown in Table 6. Two samples confirmed positive for oxycodone with all other opiates being confirm in only one sample.

Table 6 Confirmatory testing results for opiates detected in blood.

Analyte	Number of Positive Samples	Average Concentration (ng/mL)	Standard Deviation	Range (ng/mL)
Oxycodone	2	63	N/A	61-66
Tramadol	1	N/A	N/A	28
Methadone	1	N/A	N/A	<50
Hydrocodone	1	N/A	N/A	28
Morphine	1	N/A	N/A	130
Oxymorphone	1	N/A	N/A	19

Cannabinoids – The confirmatory testing results for cannabinoids, including THC, THC-OH, and THC-COOH are shown in Table 7. Forty-one samples (71%) of the 58 samples that were sent for confirmatory testing were confirmed positive for THC and/or its metabolites THC-OH and THC-COOH. Seventeen samples (29%) were positive only for the metabolite THC-COOH. Five of the samples (9%) containing THC-COOH were at concentration less than the limit of quantitation and were not included in the reported average concentration, standard deviation or range.

Table 7 Confirmatory testing results for cannabinoids detected in blood.

Analyte	Number of Positive Samples	Average Concentration (ng/mL)	Standard Deviation	Median (ng/mL)	Range (ng/mL)
THC-COOH	41†	24	±18	21.4	5.3-82
THC	24	4	±6	1.4	1.1-28
THC-OH	2	47	N/A	47.7	8.4-87

†Five sample concentrations less than limit of quantitation (5 ng/mL): results not included in average concentration, standard deviation, or range.

Urine Samples

Alcohol – A total of 226 urine samples were examined for the presence of alcohol. One hundred and fifty of the urine samples or 66% of the population were negative for alcohol. Seventy-six urine samples or 34% of the population were positive. With respect to the urine positive alcohol samples (n=76), 50% of the samples were positive for alcohol only, followed by 28% being positive for alcohol and multiple drugs (2 or more), and 20% were positive for alcohol plus one drug (Note: Two samples were not included in the totals because they contained ethanol in addition to dimethylone and methylone, and it could not be determined if the drugs were co-ingested or if dimethylone had been ingested and metabolized to methylone). For the cases with a single drug plus alcohol the drug results were as follows: THC (n=6), cocaine (n=2), methamphetamine (n=1), amphetamine (n=1), alpha-PVP (n=1), ethylone (n=1), ketamine (n=1), tramadol (n=1) and amantadine (n=1).

Drug Screening –

Immunoassay – A total of 225 urine samples (2 samples were not tested due to limited sample volume) were screened via immunoassay. Forty-three percent of those samples screened positive for THC, followed by cocaine at 15% and MDMA at 8%. No samples screened positive for 6-monoacetylmorphine (6-MAM) or synthetic cannabinoids (JWH018-COOH). The confirmation rate of the screening results was at least 90% for all compounds with the exception of methamphetamine (82%) and benzodiazepines (47%).

RapidFire tandem mass spectrometry – All 225 urine samples available for testing were screened using a targeted analysis for designer stimulant compounds as well as synthetic cannabinoids. Results are shown in Table 8. Of the samples that produced a

quantitation value above 10 ng/mL on the screen, 15 of 17 (88%) of the methylone screen positives were confirmed followed by 60% of butylone/ethylone/dimethylone samples and 60% of alpha-PVP (Table 8). Samples that screened positive for 25I-NBOMe, α-PPP, JWH018-COOH, and/or JWH-073 were not confirmed.

Table 8 Positive urine screening results for samples analyzed by RapidFire tandem mass spectrometry.

	Analyte						
	Methylone	**Alpha-PVP**	**Butylone/ Ethylone/ Dimethylone**	**25I-NBOMe**	**Alpha-PPP**	**JWH018-COOH**	**JWH073-COOH**
# of Positive Samples	17	5	15	2	6	4	2
# of Samples Confirmed	15	3	9	1	0	0	0

GC/MS base screen – A total of 225 urine samples were screened for basic compounds via gas chromatography/mass spectrometry. The highest percentage of screen positive results was for amphetamines (23%) followed by NPS (21%). Drugs listed in the "other" category included the following: diphenhydramine, aripiprazole, azithromycin, chlorpheniramine, acetaminophen, citalopram, carbamazepine, antipyrine/phenazone, bupropion, dextromethorphan, fluoxetine, methylphenidate, psilocin, tramadol, methadone, ketamine/norketamine, quetiapine, buprenorphine, and norbuprenorphine.

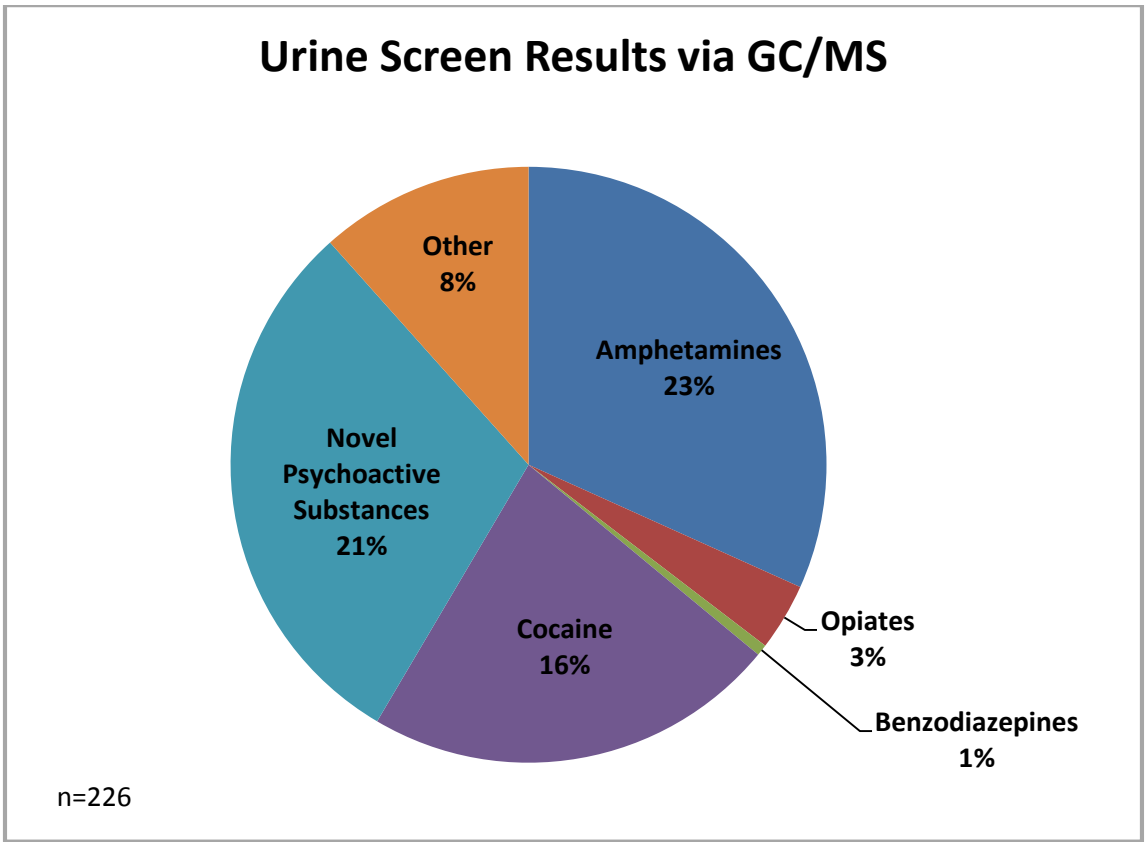


Figure 5 Urine screening results by drug class as determined by gas chromatography mass spectrometry.

TOF screen – All 2014 urines and selected 2015 years (n=127) were screened via liquid chromatography time-of-flight mass spectrometry (LC-TOF). Not all 2015 urines were screened via LC-TOF due to a change in the laboratory protocols for screening at AFMES. The highest percentage of screen positive results was for NPS (26%) followed by cocaine (20%). Drugs listed in the "other" category included the following: diphenhydramine, aripiprazole, azithromycin, chlorpheniramine, acetaminophen, citalopram, carbamazepine, antipyrine/phenazone, bupropion, dextromethorphan, fluoxetine, methylphenidate, psilocin, tramadol, methadone, ketamine/norketamine, quetiapine, buprenorphine and norbuprenorphine.

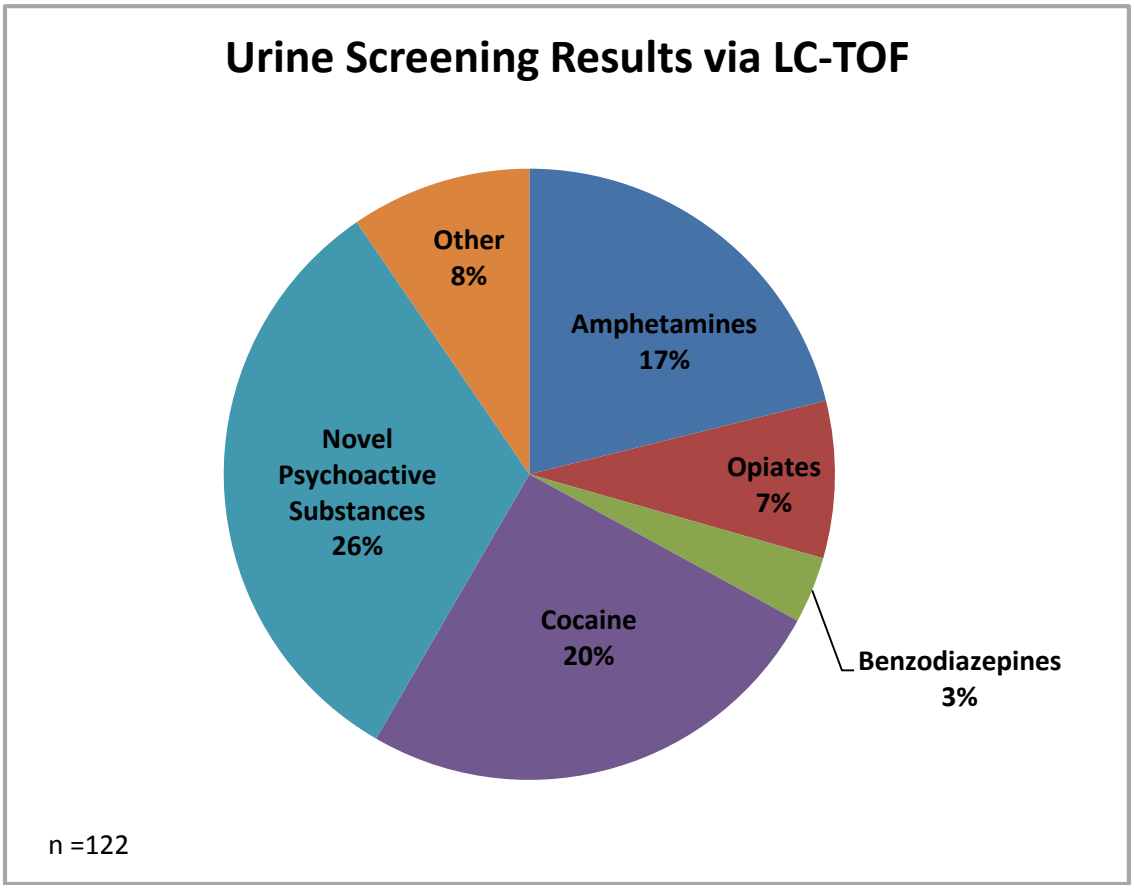


Figure 6 Urine screening results by drug class as determined by liquid chromatography quadrupole time-of-flight (QTOF) mass spectrometry.

Confirmation – The summary of confirmed results by subject are provided in Appendix D.

NPS Panel – A total of 36 urine samples screened positive on the GC/MS screen for an NPS and were subsequently confirmed. The confirmatory results for the NPS in urine are listed in Table 9, including the number of confirmed positive samples, average concentration, median concentration, and range.

Table 9 Confirmatory testing results for NPS in urine.

Analyte	Number of Positive Samples	Average Concentration (ng/mL)	Median (ng/mL)	Range (ng/mL)
Ethylone‡	18	211	540	3.7-11,971
Alpha-PVP‡	12	632	169	8.4-2,549
Butylone†	8	678	1.8	1.5-2,327
4-FA†	3	6,819	26,819	7,595-46,042
2C-B	1	60	N/A	N/A
Dimethylone†	11	1,606	704	1.9-5,369
Methylone†‡	25	6,296	842	1-91,093

†One or more sample concentrations less than limit of quantitation (1 ng/mL): results not included in average concentration, median or range calculations (4-FA n=1, butylone n=3, dimethylone n=1, methylone n=2). ‡ One or more sample concentrations greater than the upper limit of quantitation (50,000 ng/mL): results not included in average concentration, median, or range calculations (alpha-PVP n=1, methylone n=1, ethylone n=1)

Amphetamines – Thirty-three urine samples screened positive on the GC/MS for an analyte within the scope of the amphetamines confirmatory method. The confirmatory urine results for amphetamines are provided in Table 10, including the number of positive samples, average concentration, median concentration, and range.

Table 10 Confirmatory testing results for amphetamines in urine.

Analyte	Number of Positive Samples	Average Concentration (ng/mL)	Median (ng/mL)	Range (ng/mL)
MDA†‡	21	2,619	320	58-23,240
MDMA†	19	23,831	730	43-301,000
Amphetamine†	10	5,606	1,348	250-23,196
Methamphetamine†	6	18,848	1180	79-55,287
PMMA	1	<50	N/A	N/A

†One or more sample concentrations less than limit of quantitation (50 ng/mL): results not included in average concentration, median, or range calculations (amphetamine n=1, methamphetamine n=3, MDA n=2, MDMA n=3). ‡Two sample concentrations were greater than the upper limit of quantitation (2,000 ng/mL): results not included in average concentration, median, or range calculations.

Benzodiazepines – Fourteen urine samples initially produced a positive result on the benzodiazepine immunoassay screen. The confirmatory results for benzodiazepines are provided in Table 11, including the number of positive samples, average urine concentration, median concentration, and range.

Table 11 Confirmatory testing results for benzodiazepines in urine.

Analyte	Number of Positive Samples	Average Concentration (ng/mL)	Median (ng/mL)	Range (ng/mL)
α–OH Alprazolam	8	402	230	49-1,120
Alprazolam	7	182	155	69-420
7-amino clonazepam	1	743	N/A	N/A

†One sample concentration less than limit of quantitation (25 ng/mL): result not included in average concentration, median, or range calculations.

Cocaine and Metabolites – A total of 33 urine samples were sent for confirmatory testing for cocaine and/or its metabolites (benzoylecgonine and cocaethylene). The confirmatory results are for cocaine and metabolites in urine are provided in Table 12, including the number of positive samples, average concentration in urine, median concentration in urine, and range.

Table 12 Confirmatory testing results for cocaine and metabolites in urine.

Analyte	Number of Positive Samples	Average Concentration (ng/mL)	Median (ng/mL)	Range (ng/mL)
Benzoylecgonine†‡	45	11367	1304	14-155,279
Cocaine†	28	2770	197	13-24,458
Cocaethylene†	2	770	91	12-3,530

†One or more sample concentrations less than limit of quantitation (10 ng/mL: result not included in average concentration, median, or range calculations (cocaine n=7, benzoylecgonine n=4, cocaethylene n=1). ‡Two sample concentrations were greater than the upper limit of quantitation (10,000 ng/mL): results not included in average concentration, median, or range calculations.

Opiates – Eight urine samples screened positive on the GC/MS for one or more opiates within the scope of the confirmatory method. The confirmatory results for opiates in urine are provided in Table 13, including the number of positive samples, average concentration, median concentration, and range.

Table 13 Confirmatory testing results for opiates in urine.

Analyte	Number of Positive Samples	Average Concentration (ng/mL)	Median (ng/mL)	Range (ng/mL)
Oxycodone	4‡	130	130	50-210
Tramadol	2	930	930	290-1,570
Oxymorphone	2‡	89	N/A	N/A
Methadone	1	1,420	N/A	N/A
Hydrocodone	1	2,960	N/A	N/A
Hydromorphone	1	770	N/A	N/A
Morphine	1	37,300	N/A	N/A
Dihydrocodeine	1	295	N/A	N/A
Buprenorphine	1	>100	N/A	N/A
Norburprenorphine	1	48.6	N/A	N/A

‡Sample concentrations were greater than the upper limit of quantitation (3,000 ng/mL): results not included in average concentration, median, or range calculations (Oxycodone n=2, oxymorphone n=1).

Cannabinoids – Ninety-five urine samples produced a positive result for the major metabolite of THC, carboxy-THC, on the immunoassay screen and were subsequently sent for confirmatory testing. The confirmatory results, including the number of positive samples, average concentration in the urine, median concentration, and range are shown in Table 15.

Table 14 Confirmatory results for cannabinoids in urine.

Analyte	Number of Positive Samples	Average Concentration (ng/mL)	Median	Range (ng/mL)
THC-COOH	95†‡	100	62	7-818

†One sample concentration less than limit of quantitation (5 ng/mL): results not included in average concentration, median, or range calculations. ‡ 19 sample concentrations greater than the upper limit of quantitation (200 ng/mL): results not included in average concentration, median, or range calculations.

Oral Fluid Samples

Drug Screening – In 2014, a total of 122 oral fluid samples were screened in the field using the Alere® DDS2 field oral fluid drug screening device for amines (amphetamine and methamphetamine), benzodiazepines, cannabis, cocaine, and opiates. The results for each respective class are listed in Table 16.

Table 16 Results of the DDS2 screening results relative to the results obtained in the confirmatory oral fluid specimens (n=122).

	Positive	Negative	False Positives	False Negatives	Invalid
Cannabis	27	89	0	3	3
Cocaine	12	107	0	0	3
Amphetamine	3	118	0	0	1
Methamphetamine	1	117	1	0	3
Benzodiazepines	1	120	0	0	1
Opiates	0	119	0	0	3

For cocaine, amphetamine, benzodiazepines and opiates, there were no cases in which a result produced for one of the target drug classes by the device was not confirmed in the laboratory based oral fluid test (i.e. false positive). To determine false negatives, the results of the DDS2 were compared to the results of the additional oral fluid sample generated via LC-QTOF (All oral fluid samples were confirmed for cannabis). This resulted in 100% sensitivity, specificity and accuracy for each of those drug classes. For cannabis, there were three cases where a positive result was produced by the device, but detected in the confirmatory specimen, resulting in 90% sensitivity and 97.4% accuracy. There was one case where a positive result was produced on the device for methamphetamine, but methamphetamine was not detected in the confirmatory specimen. The overall sensitivity, specificity and accuracy of the device were 93.6%, 99.8%, and 99.3%, respectively. Keep in mind however that the device does not test for NPS drugs.

Full validation data for the liquid chromatograph time-of-flight mass spectrometer can be found in Appendix C. The within run and between run ppm error and retention time error as well as average reported response of all runs were tabulated. The ppm error had to be <5 relative to the reported accurate mass in the library and retention time had to be within ±0.25 minutes of the reported library retention time as well as a response of greater than 800. All of the oral fluid controls (low, mid and high) run in triplicate across the three days met all acceptance criteria. For the autosampler stability assessment of the low and high controls, no sample response degraded by more than 20%, indicating the samples were stable for reanalysis up to 72 hours. All analytes were found to be stable in the matrix at -80^{o}C for up to 30 days. For the blind sample analysis (n=23), all samples that had analytes were correctly identified as true positives (n=15) as well as identifying all true negative cases (n=3). The remaining five cases contained analytes at concentrations below the cutoff to test the system and positive reporting criteria.

Three hundred and eighty-four oral fluid samples collected with the Quantisal™ were screened for both years. The highest percentage of screen positive results was for NPS (35%), which was followed by cocaine where 86 samples initially screened positive for cocaine (22%). Drugs listed in the "other" category included the following: diphenhydramine, aripiprazole, azithromycin, chlorpheniramine, acetaminophen, citalopram, carbamazepine, antipyrine/phenazone, buproprion, dextromethorphan, fluoxetine, methylphenidate, psilocine, tramadol, methadone,

ketamine/norketamine, quetiapine and buprenorphine. The screen positive results are showing in Figure 7.

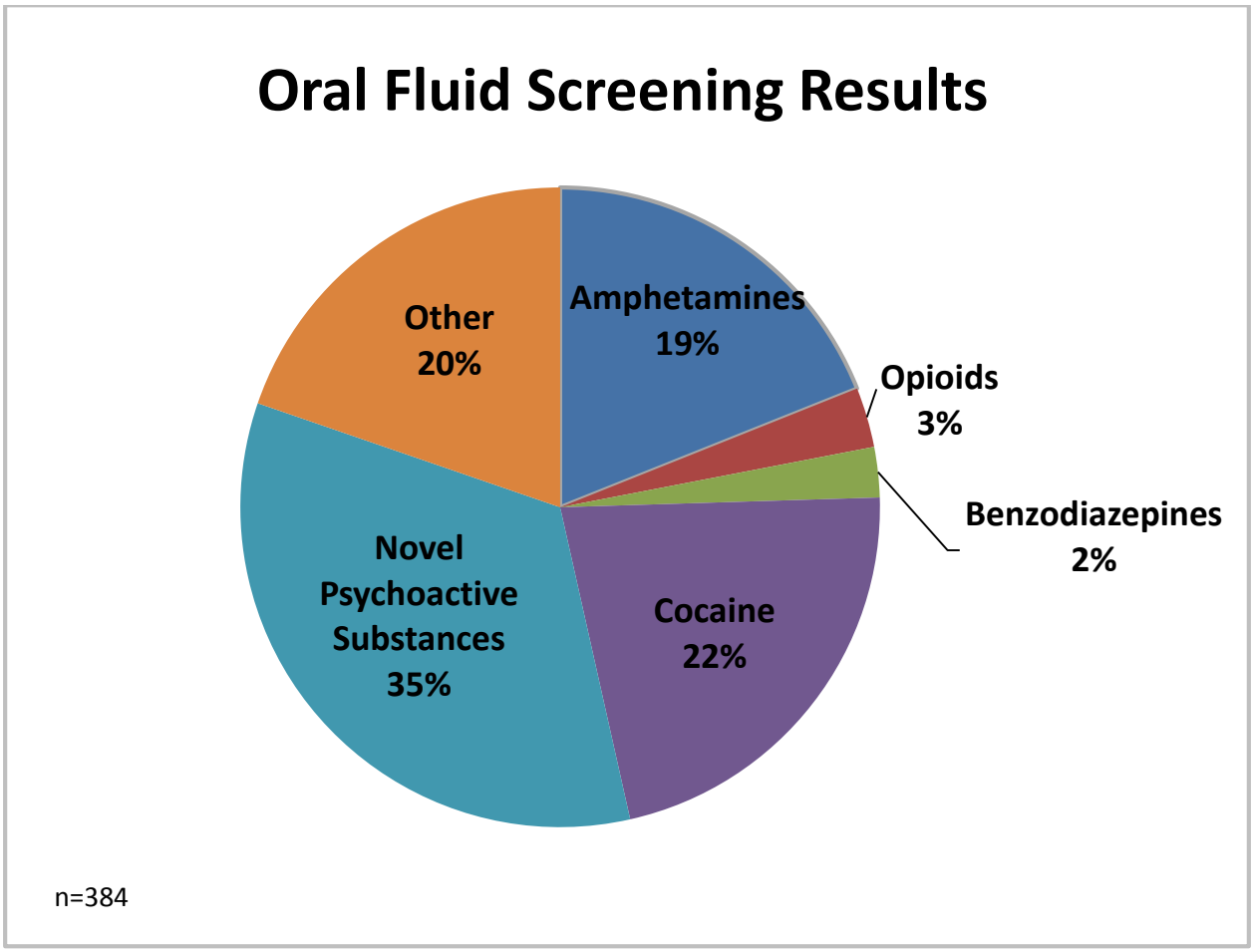


Figure 7 Oral fluid screening results by drug class as determined by liquid chromatography quadrupole time-of-flight mass spectrometry.

With respect to the 35% of oral fluid samples that screen positive for an NPS, 62 samples screened positive for ethylone/butylone/dimethylone and were sent for confirmatory testing. Shown in Figure 8 are the screen positive results specifically for the drugs within the NPS category.

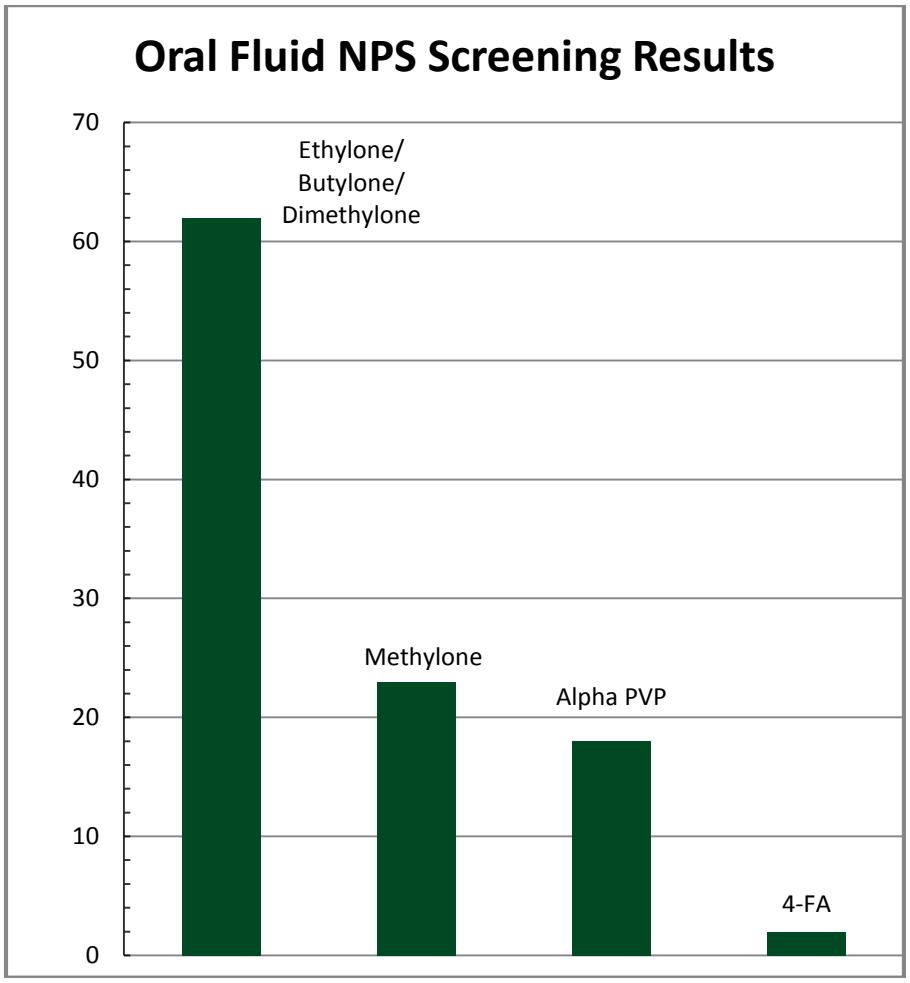


Figure 8 Number of oral fluid samples that screened positive for an NPS compound.

Confirmation

NPS Panel – A total of 78 oral fluid samples were sent for confirmatory testing for NPS. The results for the confirmatory testing are reported in Table 17. Thirty-nine percent of the samples produced a result above the limit of quantitation.

Table 17 Confirmatory result for NPS in oral fluid.

Analyte	Number of Positive Samples	Average Concentration (ng/mL)	Standard Deviation	Median (ng/mL)	Range (ng/mL)
Ethylone	56*	582	±782	335	41-4,105
Methylone	24*	2445	±3084	1,154	40-10,027
Alpha-PVP	12*	474	±566	254	86-1,301
Dimethylone	7*	611	N/A	N/A	N/A
Butylone	4*	497	±272	412	175-905
4-FA	2	329	±68	329	281-378

* Positive number of samples includes samples that had a result less than the limit of quantitation, but were determined to be positive based on a chromatographic peak at correct retention time and passing ion ratios (alpha-PVP n=8, butylone n=1, dimethylone n=6, ethylone n=17, and methylone n=7).

Oral Fluid Qualitative Confirmation Panel – A total of 95 of the 384 oral fluid samples screened positive for one or more compounds outside the scope of the NPS confirmatory method and were sent for a qualitative confirmation. The results are shown in Table 18. Approximately 48% of the samples that were sent for confirmation were positive for cocaine and/or its metabolite benzoylecgonine, which was followed by 32% of the sample being positive for MDMA and/or MDA.

Table 18 Confirmatory results for compounds outside the scope of the NPS and THC methods in oral fluid.

Class	Analyte	Number of Positive Samples
Benzodiazepines	Alprazolam/α-OH-Alprazolam*	2
	Lorazepam	2
	Clonazepam	1
Amphetamines	MDMA/MDA*	14
	MDMA	13
	Amphetamine	7
	Methamphetamine	6
	MDA	3
Cocaine	Cocaine/Benzoylecgonine*	34
	Cocaethylene	12
	Benzoylecgonine	7
	Cocaine	5
Opiates	Oxycodone	3
	Morphine	2
	6-Monoacetylmorphine	1
	Hydrocodone	1
Other Compounds	Dextromethorphan	1
	Dehydronorketamine/Norketamine/Ketamine†	1
	Citalopram†	1

*Positive sample contained both parent and metabolite.
†Oral fluid samples screened positive for dehydronorketamine, norketamine, and/or ketamine and citalopram, but these analytes are not within the scope of the confirmatory method.

THC – All 384 oral fluid samples were sent for confirmatory testing for THC. Of the 384 subjects who provided oral fluid samples, THC was detected in 152 (39.5%) subjects, with THC being over 2 ng/mL in 131 (34.1%) subjects. THC concentrations were between the limit of detection and limit of quantitation in 5.4% of the positive cases. The results for the confirmatory testing are reported in Table 19. 110 (28.4%) subjects were positive for THC at a threshold of 5 ng/mL, 82 (21.3%) at a threshold of 10 ng/mL, 73 (19.0%) at a threshold of 15 ng/mL, and 57 (14.8%) at the threshold of 25 ng/mL.

Table 19 Confirmatory results for cannabinoids in oral fluid.

Analyte	Number of Positive Samples	Average Concentration (ng/mL)	Standard Deviation	Median	Range (ng/mL)
THC	152†	64	±118	20	2-857

†21 sample concentration less than limit of quantitation (2 ng/mL): results not included in average concentration, standard deviation, or range calculations.

Synthetic Cannabinoids (*JWH-018, AM-2201, JWH-081, JWH-122, JWH-210, UR-144, XLR-11, AB-FUBINACA, ADBICA, 5F-ADBICA, ADB-PINACA, ADB-FUBINACA, 5F-ADB-PINACA, JWH-018 Adamantyl, 5F-JWH-018 Adamantyl Carboxamide, JWH-018 Adamantyl Carboxamide, PB-22, AKB-48, 5F-PB-22, 5F-AKB-48, BB-22, AM-2201 Benzimidazole, THJ-2201, THJ-018, 5F-AB-001, AB-PINACA, AB-CHMINACA*) **–** All of the 2015 oral fluid samples (n=248) were negative for the presence of synthetic cannabinoids within the scope of the method.

Oral Fluid to Blood Ratios – With respect to oral fluid to blood ratios, there is limited data in the literature comparing blood to oral fluid test results, especially for NPS. Samples with a paired blood and oral fluid were used to compare concentrations between the two matrices for NPS. The number of subjects with paired blood and oral fluid samples were as follows: methylone (n=5), alpha-PVP (n=4), ethylone (n=2), 4-FA (n=1) and dimethylone (n=1).

Methylone – The results for the paired sample blood and oral fluid concentrations for methylone as well as the average concentration for each fluid are shown in Table 20.

Table 20 Comparison of blood and oral fluid concentrations for methylone.

Subject ID	Blood Concentration	Oral Fluid Concentration	OF/Blood Ratio
MS006	168 ng/mL	1304 ng/mL	7.76
MS064	29 ng/mL	652 ng/mL	22.48
MS065	28 ng/mL	284 ng/mL	10.14
MS124	375 ng/mL	7169 ng/mL	19.11
MS329	9 ng/mL	40 ng/mL	4.44
Average	**121 (±155) ng/mL**	**1890 (±2989) ng/mL**	**12.79 (±7.68)**

The oral fluid versus blood concentration scatter plot for methylone is shown in Figure 9. In comparing the five samples, there was a correlation (R^2=0.92); however, with a limited number of samples the correlation could easily be skewed. Additionally, there were two samples that had similar blood concentrations, 28 and 29 ng/mL respectively, that produced variable oral fluid concentrations of 652 and 284 ng/mL, resulting in oral fluid ratios that differed approximately by a factor of 2 (22.48 compared to 10.14).

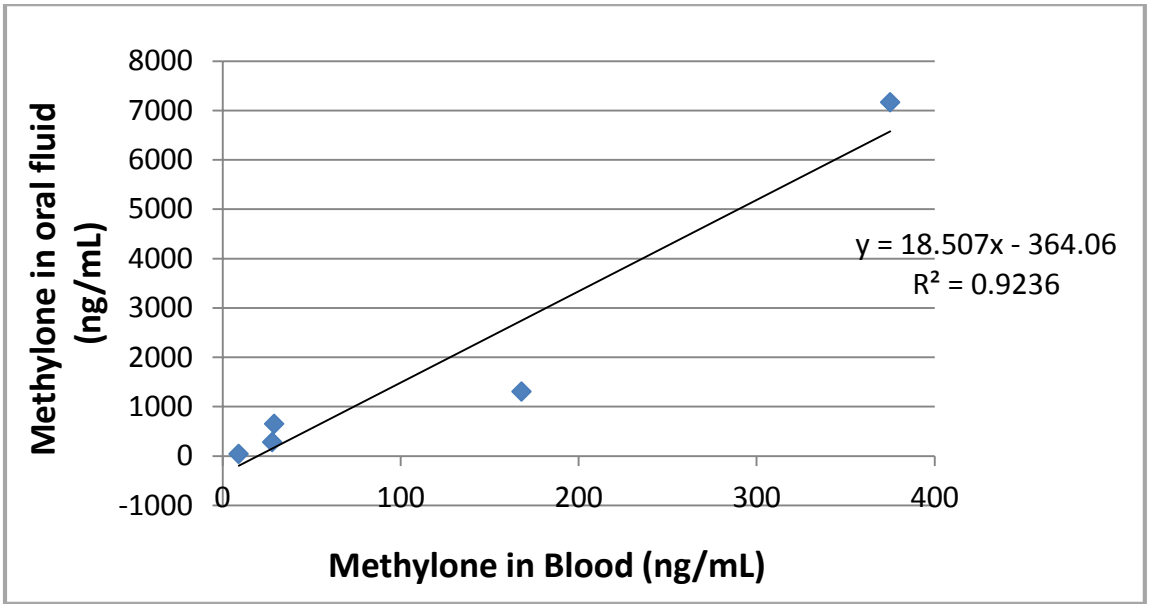


Figure 9 Scatter plot comparison and correlation coefficient of methylone concentrations in blood and oral fluid.

Alpha-PVP – The results for the paired sample blood and oral fluid concentrations as well as average concentrations for each fluid for alpha-PVP are shown in Table 21.

Table 21 Comparison of blood and oral fluid concentrations for alpha-PVP.

Subject ID	Blood Concentration	Oral Fluid Concentration	OF/Blood Ratio
MS075	80 ng/mL	379 ng/mL	4.73
MS107	10 ng/mL	128 ng/mL	12.80
MS109	8 ng/mL	87 ng/mL	10.87
MS124	87 ng/mL	1301 ng/mL	14.95
Average	**46±43 ng/mL**	**473±566 ng/mL**	**10.84±4.39**

The oral fluid versus blood concentration scatter plot for alpha-PVP is shown in Figure 10. For the four samples represented, there was a not a strong correlation (R^2=0.62). As was the case with methylone, there were two samples that had similar blood concentrations, 80 and 87 ng/mL respectively, that produced variable oral fluid concentrations of 379 and 1301 ng/mL resulting in oral fluid ratios that differed approximately by a factor of 3 (4.73 compared to 14.95).

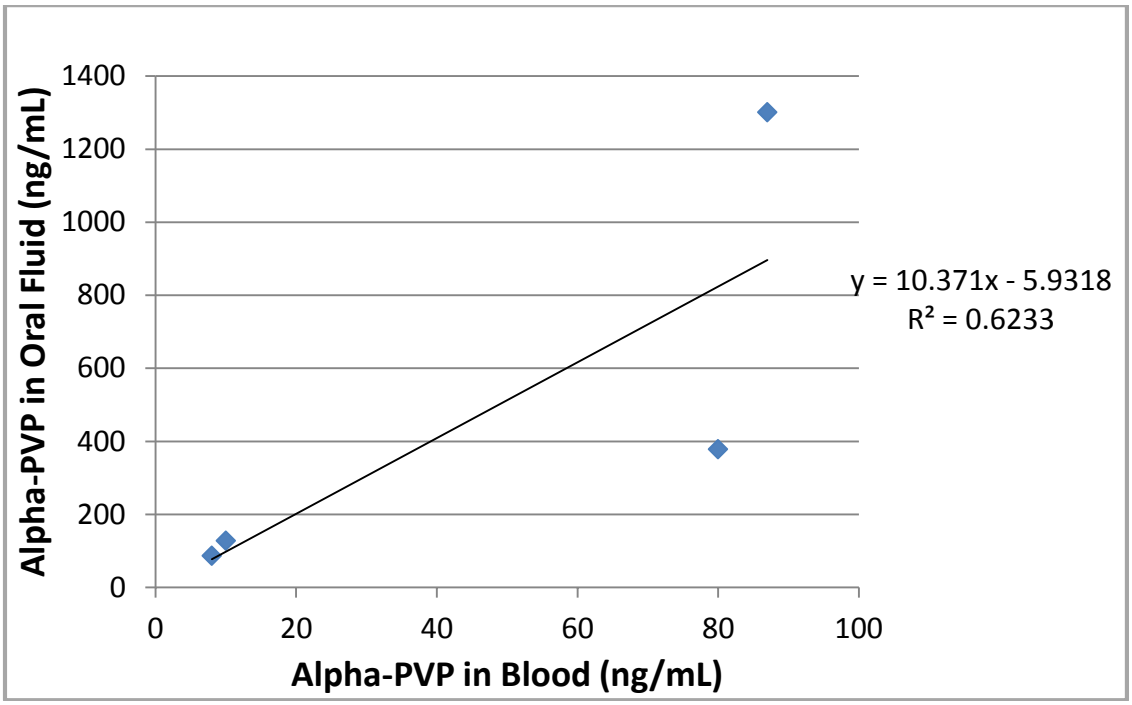


Figure 10 Scatter plot comparison and correlation coefficient of alpha-PVP concentrations in blood and oral fluid.

Ethylone – There were two paired blood and oral fluid samples, which were confirmed for ethylone. The resulting oral fluid and blood concentrations are reported in Table 22. Due to the limited number of paired samples, a correlation scatter plot could not be generated.

Table 22 Comparison of blood and oral fluid concentrations for ethylone.

Subject ID	Blood Concentration	Oral Fluid Concentration	OF/Blood Ratio
MS175	210 ng/mL	584 ng/mL	2.78
MS329	212 ng/mL	728 ng/mL	3.43

4-FA – There was one paired blood and oral fluid sample, which were confirmed for 4-FA. The resulting oral fluid and blood concentrations are reported in Table 23. Due to the limited number of paired samples, a correlation scatter plot could not be generated.

Table 23 Comparison of blood and oral fluid concentrations for 4-FA.

Subject ID	Blood Concentration	Oral Fluid Concentration	OF/Blood Ratio
MS006	71 ng/mL	378 ng/mL	5.32

Dimethylone – There was one paired blood and oral fluid sample, which were confirmed for dimethylone. The resulting oral fluid and blood concentrations are reported in Table

24. Due to the limited number of paired samples, a correlation scatter plot could not be generated.

Table 24 Comparison of blood and oral fluid concentrations for dimethylone.

Subject ID	Blood Concentration	Oral Fluid Concentration	OF/Blood Ratio
MS064	153 ng/mL	511 ng/mL	3.33

The oral fluid to blood ratios described above present some of the first reported ratios for NPS, however, with the limited number of paired samples definitive conclusions regarding oral fluid to blood ratios cannot not be determined. Uniformly, the NPS concentrations were higher in oral fluid than in the corresponding blood samples with oral fluid to blood ratios ranging from 2.78 to 22.48.

Oral Fluid THC compared to Blood THC

Of the 125 subjects who provided both blood and oral fluid samples, 73 (58.4%) were negative for THC in both blood and oral fluid, and 21 (16.8%) were positive for THC in both blood and oral fluid. An additional 13 subjects with positive oral fluid THC concentrations had blood samples positive for THC metabolites, bringing confirmed positives for marijuana use to 34 (27.2%). Ten subjects (8%) were positive for THC in oral fluid, but negative for any cannabinoids in blood. In these 10 cases, the oral fluid THC concentrations ranged from <2 to 511.8 ng/mL (mean 63.5, median 4). Three subjects (2.4%) were positive for THC in blood but negative in oral fluid (range 1.3 to 28, mean 10.2, and median 1.3 ng/mL). In comparison, the correlation between blood and oral fluid concentrations was poor (R2=0.028), with oral fluid to blood ratios (OF/B) ranging from 0 to 279 (mean 25.5, median 1.9) (Table 25 and Figure 11).

Table 24 Comparison of blood and oral fluid concentrations for THC.

Sample	OF (ng/mL)	Blood (ng/mL)	Ratio OF/Blood
MS009	7.3	1.2	6.08
MS012	105.4	13	8.11
MS014	307.1	1.1	279.18
MS015	7.9	6.4	1.23
MS048	9.6	5.1	1.88
MS049	6.7	11	0.61
MS064	25.4	1.7	14.94
MS065	42.3	1.3	32.54
MS075	5.4	2.7	2.00
MS107	111	1.3	85.38
MS108	60.9	1.8	33.83
MS109	207.3	3.1	66.87

MS111	3.8	2.3	1.65
MS123	10	3.9	2.56
MS124	93	1.4	66.43
MS236	4.9	1.3	3.77
MS045	0	1.3	*0.00*
MS155	0	1.3	*0.00*
MS266	0	28	*0.00*

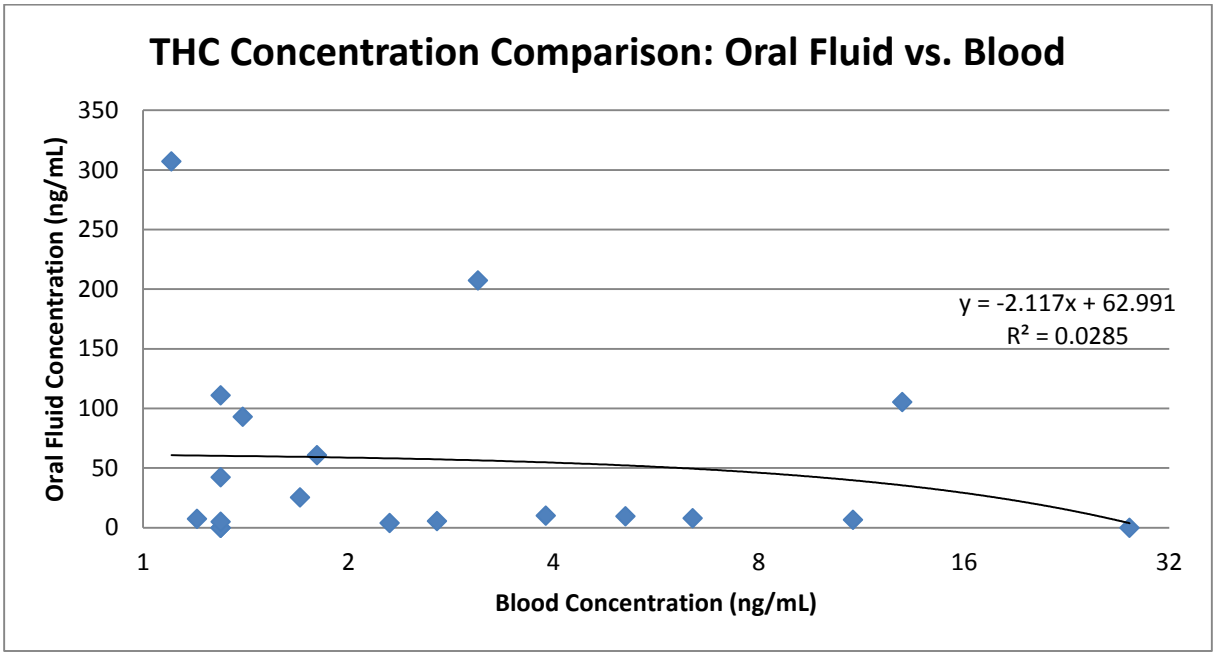


Figure 11 Scatter plot comparison and correlation coefficient of THC concentrations in blood and oral fluid.

Of these 125 paired blood and oral fluid samples, 28 (22.4%) samples were positive for THC at a threshold of 5 ng/mL, 19 (15.2%) at a threshold of 10 ng/mL, 16 (12.8%) at a threshold of 15 ng/mL, and 14 (11.2%) at a threshold of 25 ng/mL. Using these positivity rates at varying cutoff concentrations, the oral fluid THC concentration/cutoffs required to produce a positivity rate equal to that of THC in blood at a 1ng/mL cutoff (19.2%) was 7.2 ng/mL. Using an oral fluid cutoff of 2 ng/mL increased the positivity rate to 27.2%. The data confirms the poor correlation between quantitative values for THC in blood and oral fluid in this population of recreational drug users, and suggests that more sensitive cutoffs based on analytical capability rather than physiological ratios will correctly identify the greatest number of drug using subjects.

Year-over-Year changes in positivity in Oral Fluid

One of the goals of this study was to use the cohort as a monitor for trends in NPS demographics over time. Table 26 shows the relative positivity of drugs detected in oral fluid samples in 2014 and 2015.

Table 26 Oral fluid positivity rates compared between 2014 and 2015.

Analyte	2014 Oral Fluid Positivity	2015 Oral Fluid Positivity
THC	41%	37%
Methylone	18%	0.5%
Cocaine/Benzoylecgonine	13%	11%
Ethylone	11%	17%
Alpha-PVP	9%	0%
MDMA/MDA	7%	8%
Amphetamine/Methamphetamine	4%	2%
Dimethylone	4%	0.5%
4-FA	1.5%	0%

Comparing the relative positivity of drugs detected in oral fluid samples in 2014 and 2015, the rate was consistent for THC and MDMA/MDA. However, for methylone and alpha-PVP, there was a sharp decrease in the positivity rates in 2015 compared to 2014 (18% compared to 0.5% for methylone and 9% compared to 0% for alpha-PVP). With the decrease in positivity for these two drugs, there was an increase in positivity for ethylone from 11% to 17%, suggesting the market had shifted in terms of relative prevalence and popularity of NPS.

Metabolite Identification

Alpha-PVP

Through examination of the data from the urine and blood samples, alpha-PVP was seen to undergo extensive phase I metabolism, and eight phase I metabolites were identified in the *in vitro* assays with HLMs. The metabolic pathways observed were hydroxylation of the side chain, at each of two different carbons (OH-alkyl-PVP), or hydroxylation at the phenyl ring (OH-phenyl-PVP). Also seen was reduction of the ketone to an alcohol (5-OH-PVP), degradation of the pyrrolidine ring to a primary amine (amino-PVP), and oxidation of the pyrrolidine ring to a lactam (2"-oxo-PVP). 2"-oxo-PVP is further metabolized by ring opening to an unstable aldehyde, which rapidly converts to either terminal alcohol (butylamino-OH-PVP) or the corresponding carboxylic acid. The mass of the carboxylic acid is consistent with a metabolite observed (carboxyamino-PVP), but the fragmentation pattern was not sufficiently specific to confirm the detection of this metabolite. The proposed metabolic profile of alpha-PVP in humans is shown in Figure 12.

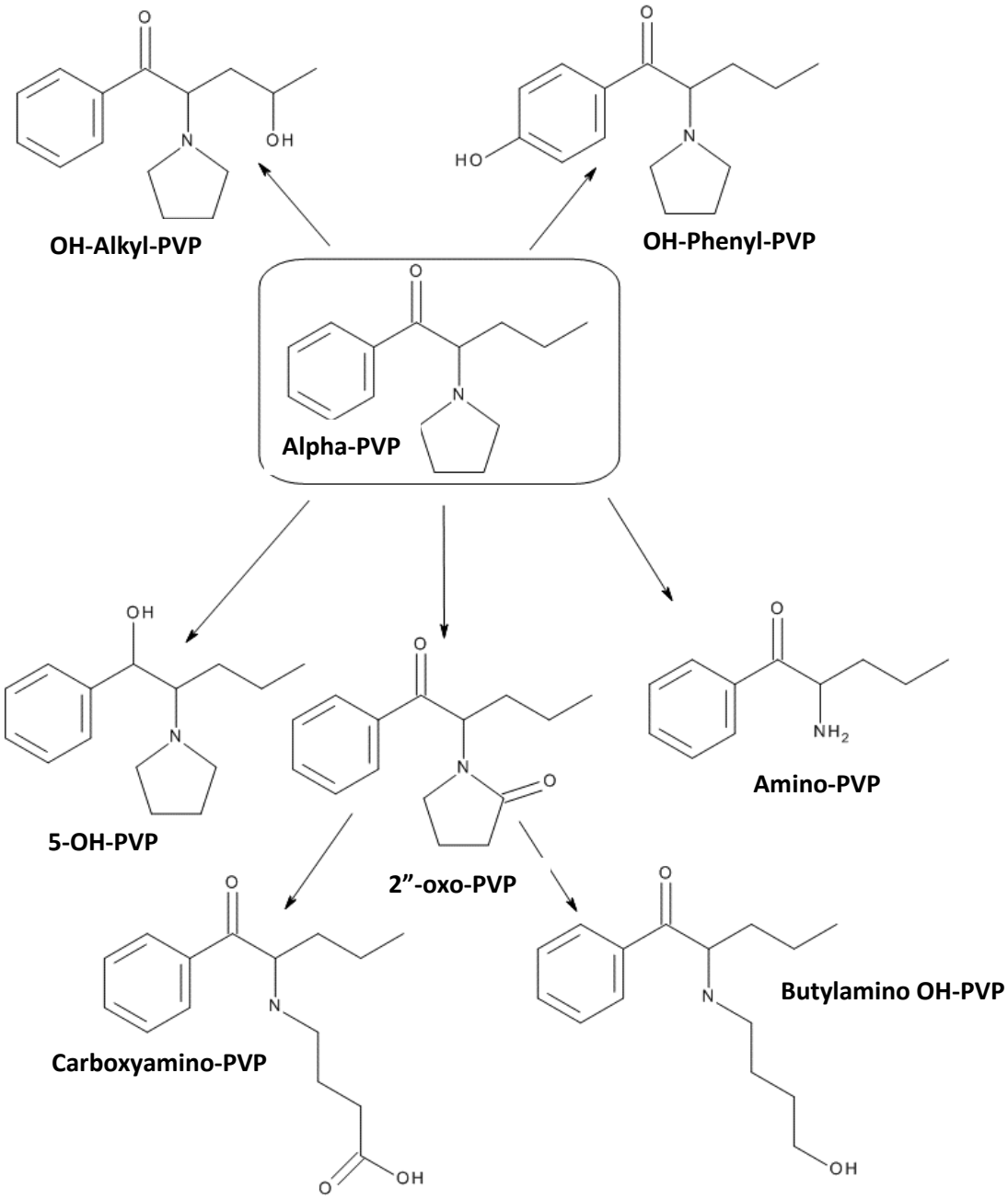


Figure 12 Proposed metabolic pathway of alpha-PVP as seen in HLM incubations.

These eight metabolites were identified using HLM incubations (5-OH-PVP, Butylamino, OH-Alkyl-PVP, 2”-oxo-PVP, OH-Alkyl-PVP, OH-Phenyl-PVP 2, OH-Phenyl-PVP 1, Amino-PVP, Carboxyamino-PVP), six of which had previously reported in rat urine and human urine. We determined an additional two metabolites in urine that were previously not detected (a positional isomer of OH-Phenyl-PVP and butylamino, OH-Alkyl-PVP).

All blood, urine and oral fluid samples were processed against the alpha-PVP metabolite library that was created in-house from the HLM experiments. A total of 126 blood samples, 226 urine samples, and 384 oral fluid samples were processed against the library.

Blood – Of the 126 blood samples, six blood samples had previously been confirmed for the parent drug. An additional four samples initially screened positive for alpha-PVP, but failed to confirm. The two major metabolites detected in the blood samples were 5-OH-PVP (n=8) and 2"-oxo-PVP (n=6). Detailed in Figure 13 are the metabolites detected in the authentic blood specimens. In the four cases that failed to confirm positive for alpha-PVP, the 5-OH-PVP metabolite was detected in two of the four cases, which would suggest alpha-PVP had been ingested, despite not detecting the parent compound.

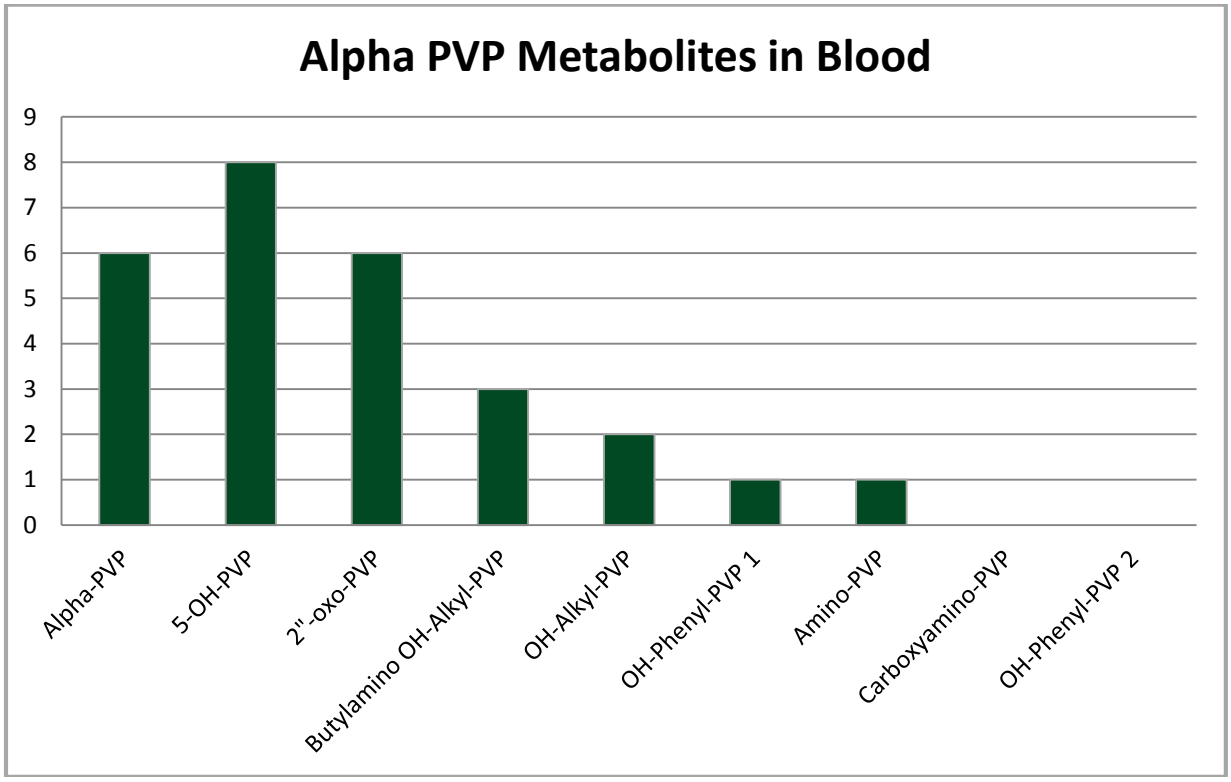


Figure 13 Alpha-PVP and the metabolites detected in authentic blood samples. The parent compound was confirmed in a total of six cases.

Urine – For the 226 urine samples, a total of 12 samples confirmed positive for the parent compound alpha-PVP. An additional sample initially screened positive for alpha-PVP, but failed to confirm. Like in the blood samples, the 5-OH-PVP (n=13) metabolite was the most commonly detected metabolite in the urine samples. However, the next most commonly encountered metabolite in the urine was metabolite butylamino-OH-alkyl PVP metabolite (n=10), followed by 2"-oxo-PVP (n=8) and OH-alkyl-PVP metabolites (n=6). Figure 14 shows the metabolites detected in the urine specimens. As expected, a more extensive metabolite profile was seen in the urine. For the case where the parent drug was not confirmed, four unique metabolites were present (5-OH-PVP, butylamino OH-alkyl-PVP, 2"-oxo-PVP and OH-alkyl-PVP).

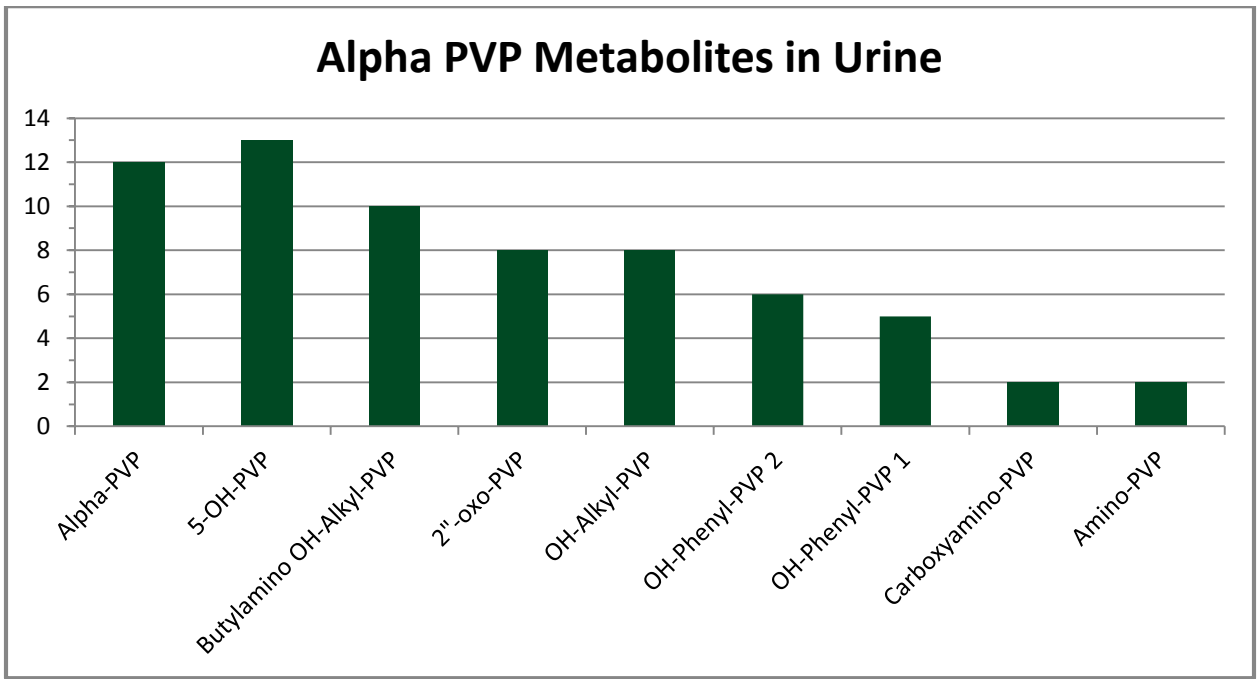


Figure 14 Alpha-PVP and the metabolites detected in authentic urine samples. The parent compound was confirmed in a total of 12 cases.

Of the 384 oral fluid samples, 12 samples confirmed positive for alpha-PVP in the oral fluid. The major metabolite identified in the oral fluid samples was the 5-OH-PVP (n=13). Shown in Figure 15 are the metabolites that were detected in oral fluid samples. In two of the samples that were confirmed positive, only the parent drug was found with no additional metabolites. An additional three samples were not confirmed for the parent drug, however, the 5-OH-PVP metabolite was detected suggesting alpha-PVP had been ingested. Two oral fluid samples showed the presence of additional metabolites that included the 2"-oxo-PVP, butylamino OH-alkyl-PVP and OH-alkyl-PVP.

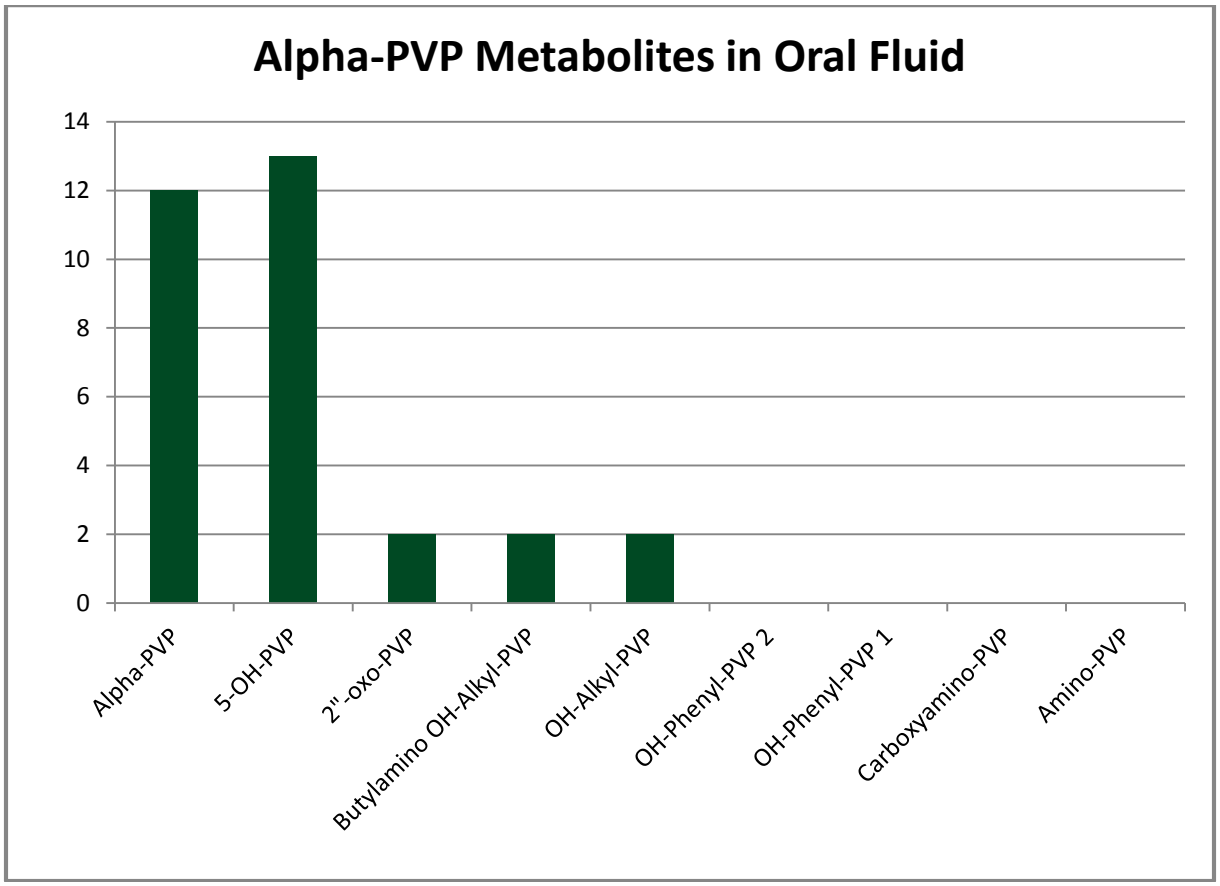


Figure 15 Alpha-PVP and the metabolites detected in authentic oral fluid samples. The parent compound was confirmed in a total of 12 cases.

The only commercially available metabolite is the 5-OH -PVP (α-Pyrrolidinopentiophenone metabolite 1, Item No. 14093 (Cayman Chemical, Ann Arbor, MI)). The standard was purchased to confirm the identity of this metabolite in the subject samples. However, comparing the standard to the authentic human samples, it was noted that while the exact mass of the compound and its fragment ions were identical to that observed in the authentic human samples, the retention time of the chromatographic peak and ion ratios of the 5-OH-PVP standard did not match what was seen in the authentic specimens (Figure 16 and 17).

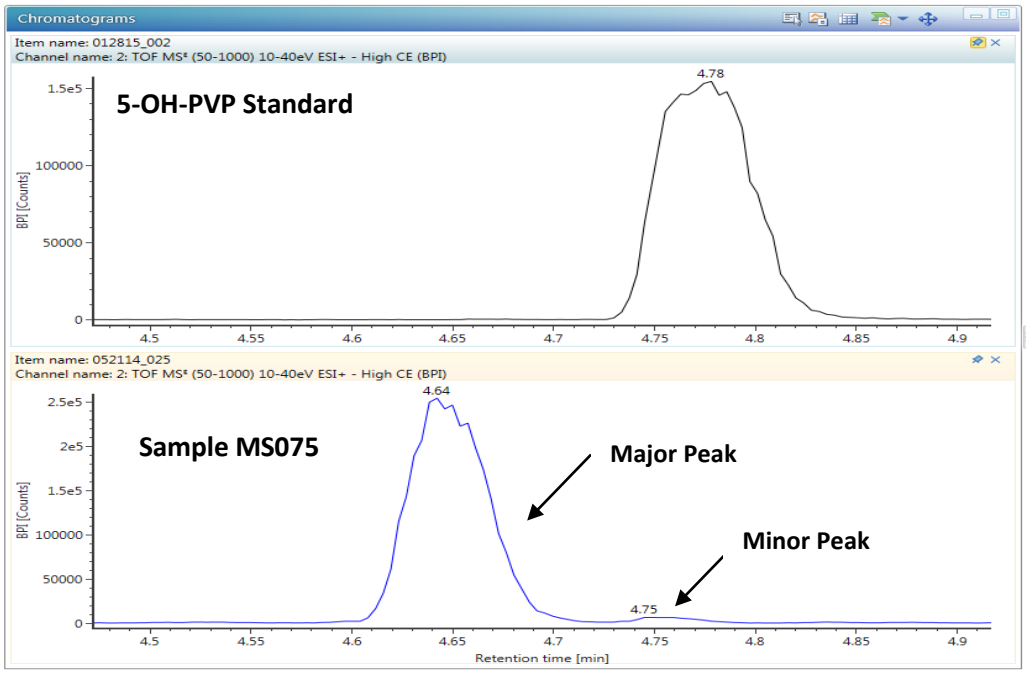


Figure 16 Chromatographic comparison of the commercially available 5-OH-PVP standard and a subject sample confirmed positive for alpha-PVP and had screen positive for the presence of the 5-OH-PVP metabolite.

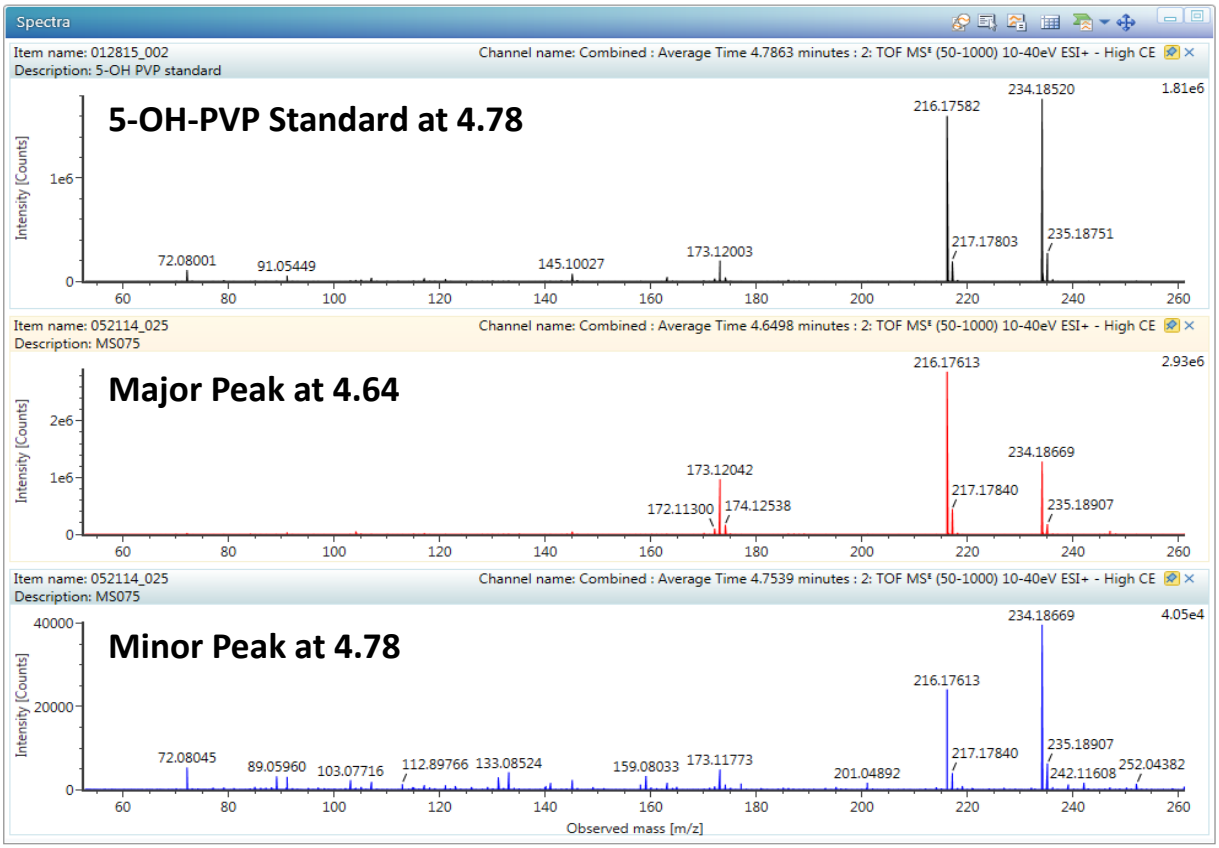


Figure 17 Mass spectral comparison of the ion ratios present in the commercially available 5-OH-PVP standard and a subject sample confirmed positive for alpha-PVP and had screen positive for the presence of the 5-OH-PVP metabolite.

The structure of 5-OH-PVP results in two adjacent chiral carbon atoms that would be expected to be excreted in urine as a mixture of diastereomers (Figure 18). We hypothesize that the commercially available standard is composed of one member of the enantiomeric pair that represents the minor metabolite, which is associated with the minor chromatographic event. The major metabolite in humans is most likely the other member of the enantiomeric pair, which is not present in the standard reference material. The configuration of the standard reference material is not provided in the manufacturer's product information

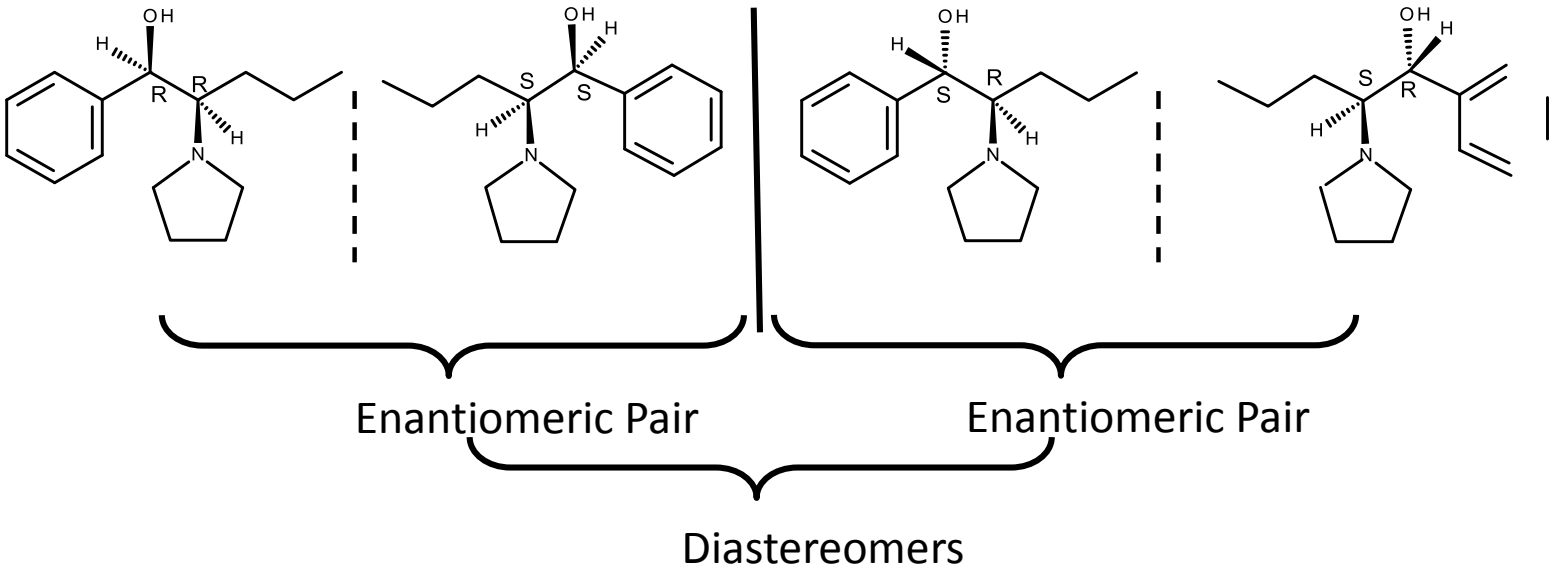


Figure 18 Structure of 5-OH-PVP depicting the two chiral carbons, which would result in a pair of diastereomers and two enantiomeric pairs.

Alpha-PVP metabolites produced using HLM were successfully identified in human blood, urine, and oral fluid samples. The primary blood metabolites include one of the 5-OH-PVP diasteromers and the 2"-oxo-PVP. This represents the first report of detecting alpha-PVP metabolites in blood and oral fluid. Moreover, we presented two blood cases and three oral fluid cases where screening only for the parent compound would have resulted in the sample being negative; however, the presence of the 5-OH-PVP would indicate prior use. Urine samples were found to contain additional metabolites with the most prevalent being the following: 5-OH -PVP, butylamino OH-Alkyl- PVP, 2"-oxo-PVP, and OH-alkyl-PVP.

Methylone/Dimethylone

Dimethylone incubations were compared to results from *in vitro* metabolism of methylone, as dimethylone was seen to metabolize into methylone by N-dealkylation, and then further by demethylenation of methylone. However, the other products of methylone metabolism (from N-dealkylation and N-hydroxylation) were not observed in the dimethylone incubations. Dimethylone also metabolized by demethylenation into 3,4-dihydroxy-N,N-dimethylcathinone followed by methylation to either 3-hydroxy-4-methoxy-N,N-dimethylcathinone or 4-hydroxy-3-methoxy-N,N-dimethylcathinone.

Although which isomer is present cannot be conclusively determined by the fragmentation pattern, previous research on the metabolism of methylone suggests that the 4-hydroxy-3-methoxy-N,N-dimethylcathinone is the primary metabolite (40-43). An additional metabolite observed has a mass consistent with the addition of one oxygen to the molecular formula of dimethylone, though the location of this hydroxylation cannot be confirmed by the fragmentation pattern. Figure 19 shows the proposed metabolic profile of dimethylone in humans. For comparison, Figure 20 gives the reported metabolic route of methylone in humans (40-43).

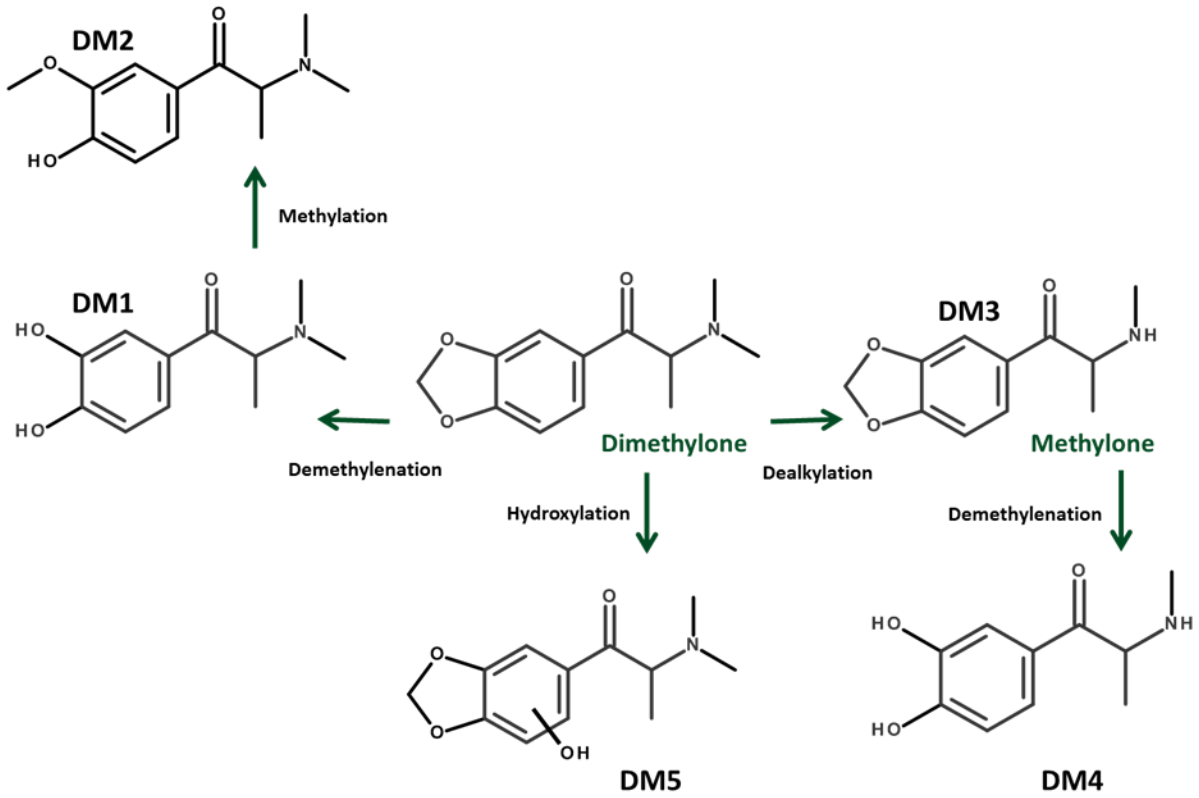


Figure 19 Proposed metabolic pathway of dimethylone as seen in HLM incubations.

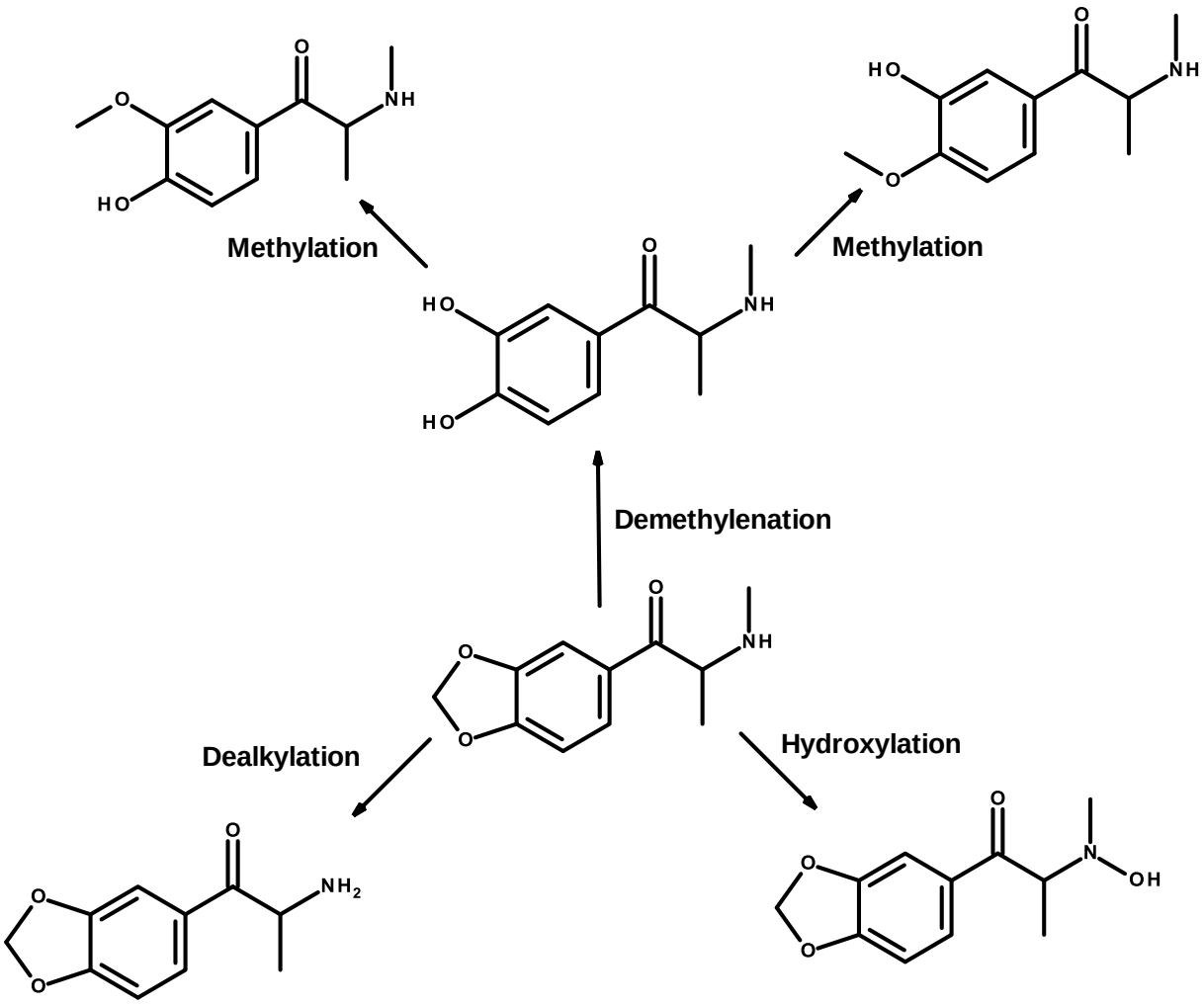


Figure 20 Previously reported metabolic pathway of methylone.

Blood – Of the 126 blood samples, five blood samples had previously been confirmed for dimethylone and methylone. The major metabolites detected in the blood samples were methylone (n=5) and the distinguishing methylated dimethylone metabolite (n=5). Detailed in Figure 21 are the metabolites detected in the authentic blood specimens. The presence cannot be confirmed if it is the product of metabolism or is present as a result of co-ingestion.

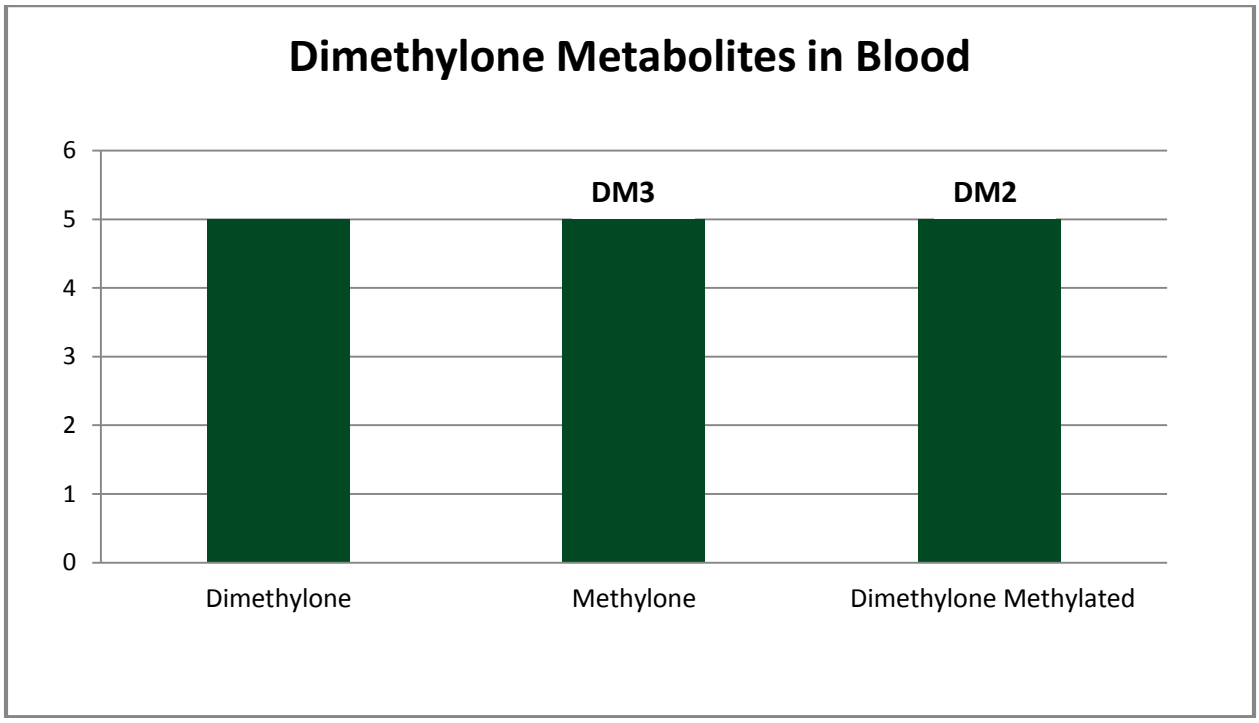


Figure 21 Dimethylone and the metabolites detected in authentic blood samples. The parent compound was confirmed in a total of five cases.

Urine – For the 226 urine samples, a total of six samples confirmed positive for dimethylone. In five of the six cases, methylone was also confirmed. The case without methylone also did not contain any other metabolites, suggesting this may have been a case with recent ingestion of dimethylone. As expected, a more extensive metabolite profile was seen in the urine. In addition to methylone, the urine samples contained hydroxylated dimethylone (n=3), methylone demethylated (n=2), and methylated dimethylone (n=1). Figure 22 shows the metabolites detected in the urine specimens.

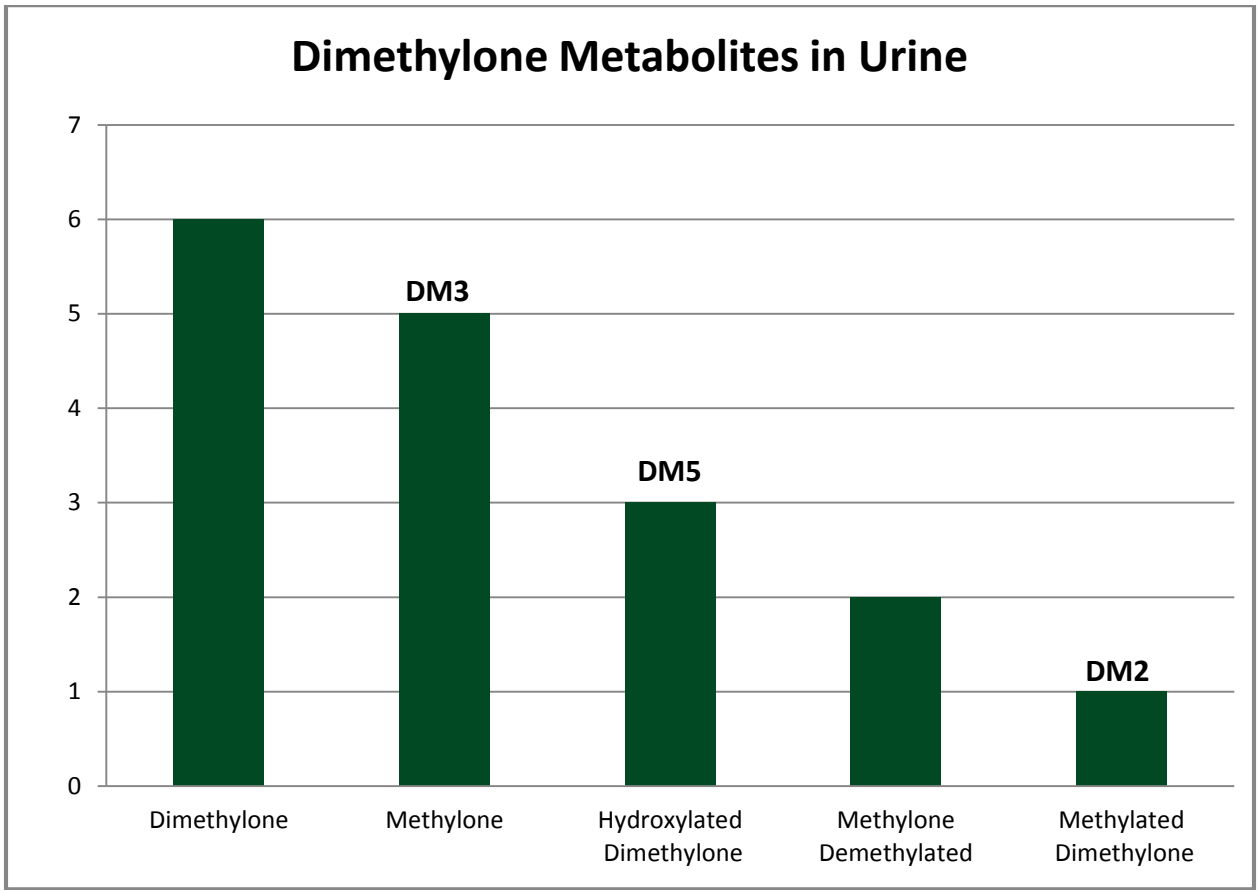


Figure 22 Dimethylone and the metabolites detected in authentic urine samples. The parent compound was confirmed in a total of six cases.

Of the 384 oral fluid samples, six oral samples had previously been confirmed for dimethylone and methylone. The major metabolites detected in the oral fluid samples were methylone (n=6) and the distinguishing methylated dimethylone metabolite (n=5). Detailed in Figure 24 are the metabolites detected in the authentic oral fluid specimens. The presence cannot be confirmed if it is the product of metabolism or is present as a result of co-ingestion.

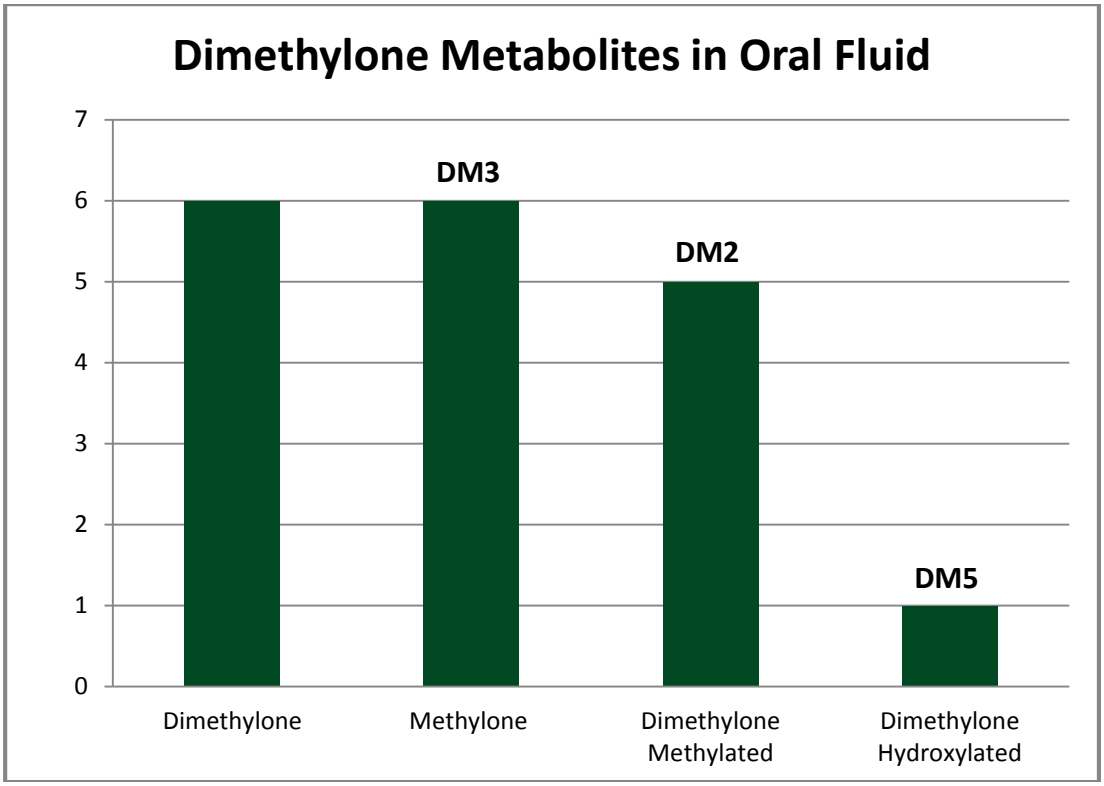


Figure 24 Dimethylone and the metabolites detected in authentic oral fluid samples. The parent compound was confirmed in a total of six cases.

Dimethylone metabolites produced using HLM were successfully identified in human blood, urine, and oral fluid samples. The main metabolite in blood and oral fluid samples was methylated dimethylone and in urine was the hydroxylated dimethylone. Generally, when dimethylone is confirmed in a sample, methylone is also confirmed. The presence of methylone in a sample cannot be definitively identified as a product of metabolism or as the result of co-ingestion. Further, due to the structural similarity between isomers (dimethylone/ethylone/butylone), the compounds follow a similar metabolic pathway and hiders the ability to identify unique metabolites because the high resolution mass spectral data is almost identical and does not provide further confirmation of which parent drug was ingested. Therefore, the co-ingestion of isomers and/or structurally similar methylone results in complex metabolic profiles.

Unidentified Analytes

Authentic human samples with additional peaks of interest were grouped to identify similarities in compound composition. Several of the original unknown peaks have masses and fragments that have been tentatively identified as metabolites either of alpha-PVP, methylone, or dimethylone based on exact mass data, fragmentation data, and retention time relative to metabolites produced *in vitro* using HLM. However, the unavailability of analytical standards for most of these potential metabolites has prevented their definitive identification, and will be the subject of future research.

Of significant interest is a peak detected in the UPLC-QTOF in authentic human samples at a retention time of 5.14 minutes (Figure 25) and on the GC/MS at a retention time of 8.38 minutes (Figure 26). This peak was identified in 10 of the 104 urine samples analyzed, all of which had detectable levels of alpha-PVP. There were only 3 additional alpha-PVP positive samples (all <70 ng/mL) that did not show this unknown peak.

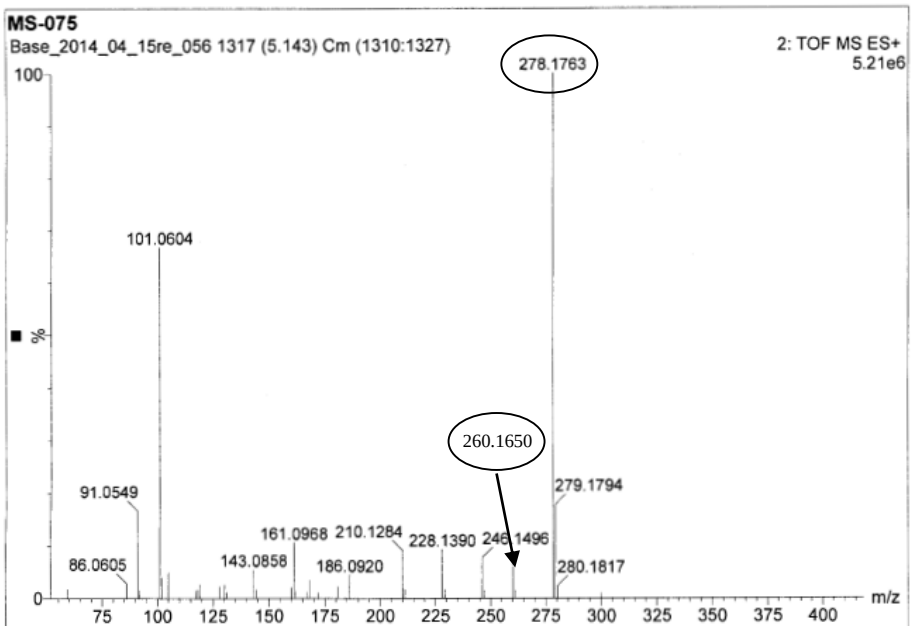


Figure 25 Fragmentation pattern from UPLC-QTOF for unknown peak (RT 5.14)

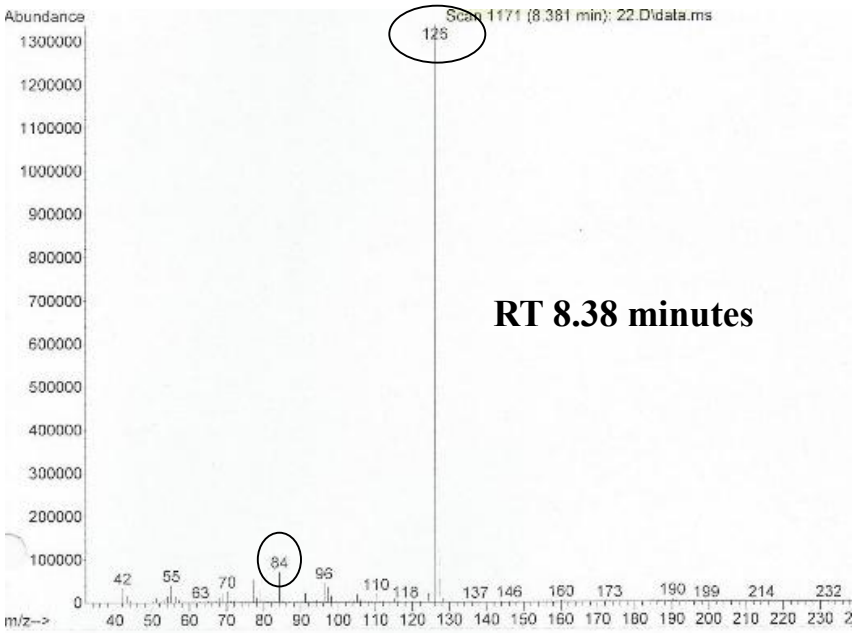


Figure 26 GC/MS Mass Spectra for unknown corresponding to UPLC-QTOF RT 5.14

The peak did not correspond to any of the potential metabolites identified in the HLM experiments. Attempts to resolve the identity of this other metabolites have included UPLC-QTOF accurate mass analysis to determine molecular formula, fragmentation and elemental composition determination, examination of the proposed mass fragmentation for several candidate compounds based on molecular formula, structural elucidation tools on the Waters UNIFI® platform, examination of the GC/MS data for the sample samples, and comparison of the spectra to those of available standard reference materials.

Once the elemental composition was determined from the exact mass data, a proposed structure and associated fragments were hypothesized (Figure 27).

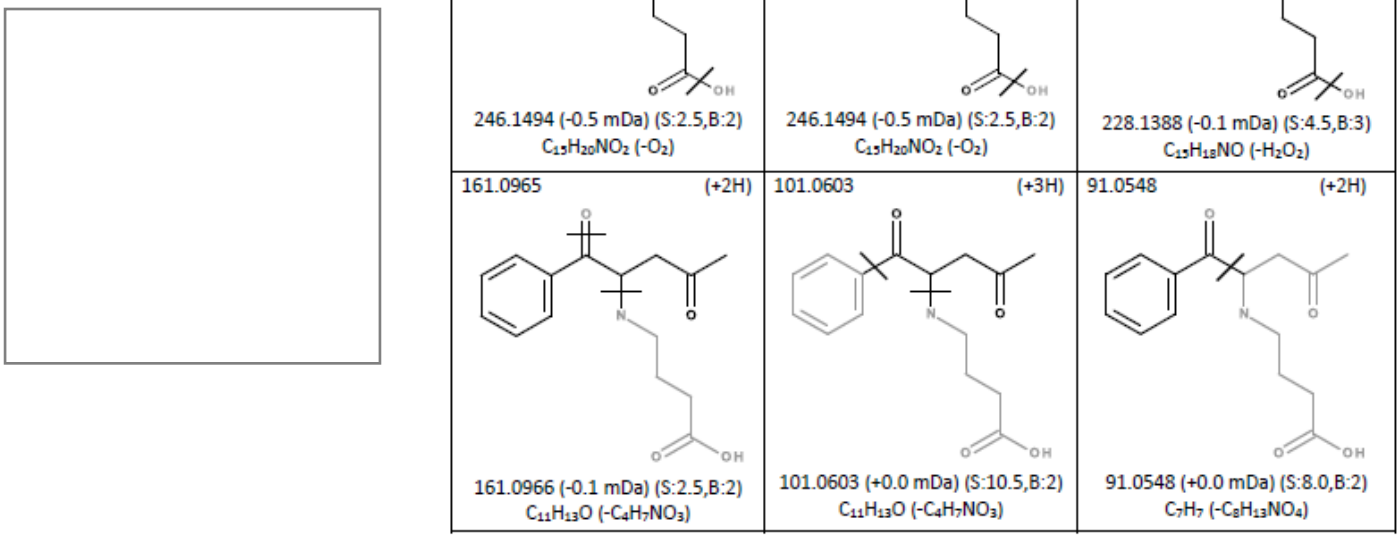
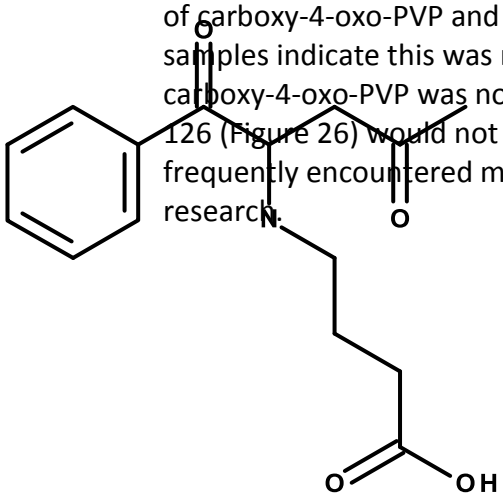


Figure 27 Proposed structure and associated fragmentation of the unknown analytes as determined by the elemental composition.

Based on the above considerations, it was initially believed this unknown was most likely to be a metabolite of alpha-PVP (carboxy-4-oxo-PVP) previously proposed by Sauer, *et. al.* (31). However, upon further investigation, the large ppm error (>130ppm) between the exact mass of carboxy-4-oxo-PVP and the exact mass of the chromatographic peak present in the human samples indicate this was not the correct identification. The GC/MS data also suggest that carboxy-4-oxo-PVP was not the correct structure since that formula cannot account for the m/z 126 (Figure 26) would not be produced from this structure. The true structure of this frequently encountered metabolite remains undetermined and will the subject of further research.



CONCLUSIONS

The objectives of this project were to develop a better understanding of four aspects of the emerging designer drug phenomenon: 1) to study and verify the reported high incidence of use of drugs, particularly emerging NPS use among attendees at EDM scene; 2) to identify the compounds of concern and their metabolites in a trio of paired biological specimens – blood, oral fluid and urine, assessing in particular the value of oral fluid as an analytical matrix for detection of these drugs; 3) to identify novel metabolites for new or emerging drugs whose metabolic fate has not been completely studied in humans, but that were identified in the cohort we studied; and 4) develop and share analytical methods and mass spectral libraries for screening and confirmation methods on various analytical platforms that would assist the forensic science community in detecting use of these drugs in investigations of criminal activity, drug use and possession, impaired driving, drug facilitated sexual assault and other violent drug related crimes.

The project has met its goals in each of these areas.

1) **Confirming High Rates of Drug Use in the EDM community:**
High rates of drug use at EDM festivals have been reported within the medical and epidemiological literature and are reflected in online forums associated with the EDM culture. Moreover, several adverse events including fatal and non-fatal drug-related overdoses have been reported at several different EDM festivals in the United States.

We developed and cultivated a novel methodology using a "living laboratory" model, identifying a representative EDM event in Miami FL, with a high attendance rate (>150,000), and appropriate attendee flow to facilitate sampling, worked with law enforcement and the Mayor's office to establish the validity of the proposed sampling event, scouted an appropriate location for subject recruitment, negotiated premium space in the heart of the event, established a temporary project command center, developed a recruiting strategy to encourage participation, engaged and trained a peer recruiting team, and deployed them to recruit subjects during the three day event over two years. Using this model, we were successful in obtaining samples of oral fluid and/or blood and/or urine from 396 subjects, along with important demographic and drug use history information.

Ultimately, study the self-reported drug use and analytical confirmation of drugs in biological specimens supported previously reported high rates of drug use within this population, especially with respect to NPS, which was the most frequently encountered drug class after cannabinoids.

Self-reported drug information collected during the survey provided the unique opportunity to compare user reports of what the subjects thought they had ingested, to what was confirmed in their biological samples.

Half the subjects who had reported using MDMA, "Molly" or Ecstasy had a biological specimen confirmed positive for an NPS, suggesting that this population is extremely vulnerable to being sold counterfeit substances which likely contain drugs different from what the user may have been expecting or have had experience or tolerance to. Many of these novel substances have significant adverse side effects. As an additional outcome of this research, making this information available to attendees at these events through drug education campaigns, warning attendees against the use of controlled substances sold at these venues, could reduce use and drug demand, and reduce the risk of some adverse events, hospitalizations and deaths.

Of the 396 subjects that participated in our study, 27% were confirmed positive for an NPS and/or MDMA in one or more biological specimens. Excluding the samples that were completely negative for drugs or alcohol (n=102), the positivity rate for an NPS and/or MDMA in drug users at the event increases to 36%. Within the population, 70 subjects (18%) were positive for NPS, excluding MDMA. With respect to the positive samples, the majority of those samples were confirmed for more than one drug, suggesting most of the drug-users within this population are poly-drug users who are at even greater risk for adverse events.

2) **Demonstrating the value of oral fluid versus blood or urine as an analytical matrix for detection of NPS and other drugs.**

Related to the biological specimens, there was good agreement between the findings in blood, urine and oral fluid with respect to the positive findings. The urine samples often contained more drugs, but this is an expected result as urine tends to retain drugs and metabolites and provide a longer detection window for indications of historical drug use compared to blood and oral fluid. Oral fluid has been demonstrated for many basic drugs to more closely mimic the time course of detection in whole blood which is more closely related to recent drug use, and we verified this to be true for NPS substances encountered in our subject pool. There was good agreement in assessing drug positivity in the subject pool between the results from blood and oral fluid samples. This provides support that oral fluid is a viable specimen for detecting recent drug use. The ease of oral fluid collection and the fact that sample collection requires noninvasive techniques adds to the overall appeal for collecting oral fluid. In comparing the blood concentrations to oral fluid concentrations, specifically for NPS, the oral fluid samples had higher concentrations of drug present supporting the use of oral fluid as an alternative to blood given the easier collection process and limited ability to adulterate the samples during the non-invasive observed collection process.

3) **Identifying novel metabolites for new or emerging drugs whose metabolic fate has not been completely studied in humans.**

Analytically the study provided valuable information about the target compounds that are most effective for detecting NPS use in individual matrices. This information has valuable implications for forensic laboratories performing this testing, depending on what samples are available to them through their local regulations and statutes. Through this project, we

optimized a robust and reliable method for producing metabolites *in vitro* using HLMs, which were used to produce proposed metabolic pathways for alpha-PVP and dimethylone as well as create metabolite libraries with these proposed metabolites. Blood, urine and oral fluid specimens were processed against these metabolite libraries to determine the relative prevalence of metabolites in the various biological matrices. For alpha-PVP, metabolites were identified in all three matrices and in some cases could be used to indicate prior ingestion in the absence of the parent drug. The primary metabolites included one of the 5-OH-PVP diasteromers and the 2"-oxo-PVP. With respect to dimethylone, the main metabolite in blood and oral fluid samples was methylated dimethylone and in urine was the hydroxylated dimethylone. Generally, when dimethylone is confirmed in a sample, methylone is also confirmed, however, the presence of methylone in a sample cannot be definitively identified as a product of metabolism or as the result of co-ingestion.

4) **Developing and validating analytical methods and mass spectral libraries for screening and confirmation methods**

Several screening approaches ranging from immunoassay, RapidFire tandem mass spectrometry, gas chromatography/mass spectrometry and a broad-based screening approach using exact mass (LCTOF) have been evaluated. Immunoassay technology is limited by the fact that most screening kits do not cross react with NPS and require specialized kits. However, by the time these kits are developed, validated and implemented the most prevalent compound have changed, and subsequent analogs of the drugs have little to no cross reactivity with the assays requiring continuous development. RapidFire tandem mass spectrometry provides high-throughput capabilities, but is relatively limited in terms of the scope. The elimination of the chromatographic component of the assay raises concerns about interference when large numbers of molecules with similar structures are the targets for the test. Due to the diversity and continual emergence of new compounds, broad-based screening using LCTOF provides the most comprehensive scope with the greatest chance of identifying emerging compounds. Limitations associated with this technology are associated with the inability to distinguish isomers with the same exact mass. In the course of the project, we developed and validated an LCTOF method for the screening for over 250 compounds, including approximately 80 NPS drugs and their metabolites. Confirmatory methods using LCMSMS for confirmation and quantitation of NPS drugs and their metabolites, as well as more traditional recreational drugs including THC were developed and validated, and applied in the quantitation of the drugs in all matrices from the target subject pool. In addition, we developed a catalog of a mass spectral data that will be shared through our website and downloadable for use by the forensic science community.

Implications for policy and practice

Based on the findings from this project, we identified implications for each of the areas addressed in the conclusions section.

1) **Confirming High Rates of Drug Use in the EDM community:**

Our work confirmed that NPS drug use in the EDM community and that NPS drugs with their entactogenic, stimulant, and empathogenic effects were an integral part of the EDM culture. After THC, the NPS drug class was the most commonly detected. Many of the drugs that are popular at these events are unscheduled, and it is difficult to control their availability, or to prosecute their distribution and sale. Until these drugs are scheduled, they do not become a routine part of the testing scope of laboratories performing testing. In turn, the Drug Enforcement Administration (DEA) and other regulatory agencies rely on the analytical data from adverse events to support their scheduling actions. This catch-22 situation impacts the effectiveness of protecting the public from the unrestricted sale of dangerous NPS drugs. The findings of this study reinforce the value of this sample collection approach in identifying market changes, especially the quick turnover with respect to the relative popularity of different NPS. At the event in 2014, the majority of samples collected were positive for methylone and alpha-PVP, however one year later, not a single case screened positive for alpha-PVP. The 2015 data suggest that the market had moved to ethylone, an isomer of methylone, but which is not currently scheduled in the United States. The data support the influence of DEA scheduling actions on the NPS market, and suggest that more rapid scheduling actions could further pressure on the market to combat availability.

Using this cohort as a sentinel population helps to keep the forensic science community up to date on what drugs are appearing and how their popularity is changing over time. This in turn helps laboratories prioritize their resources for method development and validation, rather than simply developing assays for every new drug that comes along. We have received interest in this data from emergency room staff and physicians, first responders, and public health professionals in addition to the forensic and analytical community, who wish to use the data in their professional education and drug intervention education. Collecting additional longitudinal data over time at these events will give a better perspective on the cycles with which drugs appear and disappear on the market.

2) **Demonstrating the value of oral fluid versus blood or urine as an analytical matrix for detection of NPS and other drugs.**

The data from both years of the study showed that oral fluid is an ideal matrix for large scale sample collection from a cooperative survey population. Sample collection was easy, did not require the facilities for privacy for urine collection, was observed so was not susceptible to tampering or substitution, could be accomplished in the open in less than three minutes, and did not require the use of a trained phlebotomist. Subjects were very willing to provide oral fluid samples, and felt it was much less invasive than blood draw. In addition, the oral fluid analytical results demonstrated that the positivity rate for oral fluid testing for all recreational drugs and NPS was highly correlated with the blood test results, and reflected the degree of drug use in the subjects as well as blood. The parent drug concentrations in the oral fluid were typically higher than the blood concentrations, although not quantitatively correlated. Based on this experience, we recommend that oral fluid can be used in place of blood to expedite collection from a larger sample population.

In addition to collection of oral fluid for later laboratory based screening, we evaluated on-site oral fluid testing using the Alere® DDS2 device. While the device does not currently test for NPS substances, the chemistry of the platform could be adjusted such that it would, if appropriate antibodies were available. The correlation of the field test results for commonly abused drugs, most notably THC and cocaine, were excellent.

4) Identifying novel metabolites for new or emerging drugs whose metabolic fate has not been completely studied in humans.

The approach of collecting paired blood/oral fluid and urine samples, and comparing the results of the parent drugs and metabolites identified in each with the results of human liver microsome incubations with the target drugs proved to be an effective approach for verifying the identity of the most significant markers for NPS drugs in biological samples. This will facilitate the choices of forensic laboratories in developing assays to detect illicit drug use in criminal investigations such as impaired driving and drug facilitated sexual assault. It will also assist in the identification of unknown metabolites in death investigation toxicology applications. Knowing the identity of the metabolites or markers also allows further work to be done investigating the toxicity of the drug, by being able to evaluate the metabolites for activity that may contribute to the main or side effect profile of the drugs. The experiments also provide valuable insight into the likely metabolic fate of other members of the same drug class. Using this combined approach of in vitro and in vivo drug analysis should be further exploited in understanding the adverse effects of these new drugs which will assist with federal or state drug scheduling actions.

5) Developing and validating analytical methods and mass spectral libraries for screening and confirmation methods

It is clear that immunoassay (EMIT, ELISA), which is currently used extensively for drug screening in forensic toxicology is not a realistic approach to the screening of NPS drugs. The structures are too varied to allow for significant cross reactivity, and the 12-18 month cycle time for raising antibodies developing and validating novel immunoassays would render the new tests out of date by the time they are available. Other alternatives need to be prioritized.

Our experience with the various analytical platforms suggests that high resolution mass spectrometric (HRMS) LCTOF screening for drugs suggest that has a number of advantages over traditional GCMS screening techniques, although the two work well complementarily. HRMS LCTOF still has limitations in terms of differentiating compounds with the same exact mass (isobars), however HRMS instruments have a number of analytical modes for fragmentation and data acquisition that were not fully explored in this grant. HRMS is in invaluable tool that should be made more widely available to the forensic science community for the elucidation of the identities of unknown drugs.

Implications for further research

Based on our experience with this project we identified opportunities for future research in the following areas:

1) **Sampling of drug-using cohorts as sentinel populations for emerging trends in the designer drug (NPS) market.**

Since it is now extremely difficult to ethically dose subjects with many emerging NPS drugs, especially for drug classes for which adverse side effects have been reported, we believe that opportunistic sampling of additional populations self-administering the drugs would add to our knowledge base. This would include additional testing at EDM events designed to capture both the longitudinal changes in the market demonstrated between 2014 and 2015 in the present study, and geographic differences between events in different locations in the United States. In future events we hope to also establish "amnesty bins" to secure authentic drug material to compare to the results of toxicological testing. Lessons learned from this research, including the value of oral fluid samples as an easy way to collect samples from larger groups of people, at minimal cost compared to blood sampling, would make this a more cost effective option, allowing much larger numbers of subject to be surveyed.

In addition, the subjects in this study were by their nature, and per the terms of the IRB, ambulatory, oriented, did not appear intoxicated, were over the age of 18, and attested to not having used drugs in the past 48 hours. In order to assess the major adverse effect impacts of the drugs, it would have been extremely informative to have been able to test the many individuals who were hospitalized or treated by emergency services at the event. The exact number of these is not known but during the 2014 event, one individual died as a result of his drug use, and 48 subjects were transported to an acute care hospital in downtown Miami. Collaborations developed during this study with the emergency responders and the psychiatric attending physicians in this and surrounding hospitals has created an opportunity, with IRB review already approved, to apply the oral fluid collection approach to these hospitalized patients to determine the identity of the drugs that resulted in these medical emergencies. This data would be a very valuable complement to the EDM population, and would provide very valuable data on adverse events that would assist with more rapid development of data to support emergency scheduling actions.

2) Development of NPS drug tests for point of contact oral fluid drug test devices.

The value of oral fluid testing has been demonstrated in this study by the very high degree of correlation of the oral fluid test results to the blood and urine results. We demonstrated the ease of oral fluid sample collection, and the utility of the sample in comprehensive detection of recent drug use, and were able to demonstrate the applicability of existing portable oral fluid testing devices (the Alere® DDS2) for the detection of traditional drugs of abuse. Law enforcement agencies are expressing increasing interest in portable oral fluid drug testing technology, to address the increase in DUID arrests and the increasing availability of marijuana for medical or recreational purposes. We would propose the

development of applicable chemistries on a portable oral fluid drug testing platform to enhance the sensitivity of portable oral fluid drug testing for existing drugs of concern, and the development of chemistries for these platforms for some of the more established NPS substances identified in this study (cathinones, including alpha-PVP, ethylone, methylone, dimethylone) to complement the existing tests for amphetamines, cocaine opiates and THC. Such chemistries could provide additional information to law enforcement in their investigations, and would have applications in probation and parole, jail intake, drug treatment, and the emergency room. Emerging technologies make the multiplexing and sensitivity of oral fluid testing devices much more accessible, and evaluating their applicability to the criminal justice environment would be a great benefit to those investigations. Tests developed with this goal in mind could be evaluated and validated in the EDM population as a ready source of drug positive subjects as described in the results of this report.

3) Identifying novel metabolites for new or emerging drugs whose metabolic fate has not been completely studied in humans.

In the course of this project, we identified alpha-PVP, dimethylone and ethylone as emerging NPS substances about which little was known as far as their metabolism was concerned. We made significant strides in confirming recent *in vitro* metabolomics work for alpha-PVP and identifying new information about the metabolism of dimethylone, using the same tools used to screen the human subject's samples from NPS using volunteers. Using this integrated model of *in vitro* liver microsome incubations and confirming the presence of metabolites in authentic human samples, we advanced understanding of these poorly understood metabolic pathways. We would propose continuing to use this combined complementary approach to study other emerging drugs in this and other populations, including the synthetic cannabinoids, which were not prevalent in this EDM cohort. Some websites track music events by frequency of mentions of various different types of drug use in social media posts. By selecting music, or other events (e.g. hemp fest, cannabis legalization events, etc), in which the culture favors one class of drug use over another, it would be possible to learn more about different categories of emerging drugs.

4) **Developing advanced HRMS MS^n methods to enhance the identification of unknown NPS drugs and their metabolites.**

In this study, we were successful in developing and validating efficient analytical methods using HRMS methods to identify compounds and candidate drugs by LCTOF. While this approach gave very useful information on the molecular formula of the unknown, and through tools like ChemSpider facilitated their identification, the process was somewhat labor intensive and generated multiple candidate compounds which had to be further refined by consideration of complementary analytical data such as GCMS. Current generation LCTOF technology, and associated software permits additional fragmentation of the parent compounds with accurate mass detection, and the instrument software uses intelligent algorithms to propose possible structures, making the detection and identification of novel compounds and potentially related metabolites much more

straightforward. This applies to both detection of unknown drugs and metabolites in both authentic human samples and in *in vitro* incubations.

Further work needs to be done on the application of software tools such as metabolite pilot (Sciex®), and complex data acquisition methods (SWATH technology (Sciex®)), to expedite the identification of novel drugs and their metabolites.

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DISSEMINATION OF RESEARCH FINDINGS

The data from this project has been presented at the following:

Mohr, A.L.A., Yeakel, J.K., Friscia, M., Diamond, F.X., and Logan, B.K. *A Two Year Comparative Analysis of Novel Psychoactive Substances Detected in Blood, Urine, and/or Oral Fluid within Attendees at an Electronic Dance Music Festival.* Platform presentation at the American Academy of Forensic Sciences (AAFS), Las Vegas, Nevada, February 2017.

Friscia, M., Mohr, A.L.A., Diamond, F.X., and Logan, B.K. *Development and Validation of a Confirmatory Method for Six Novel Psychoactive Substances in Whole Blood using UPLC/MS/MS*. Poster presentation at the American Academy of Forensic Sciences (AAFS) Annual Meeting, Las Vegas, Nevada, February 2017.

Krotulski, A.J., Mohr, A.L.A., Friscia, M., and Logan, B.K. *Delta-9-tetrahydrocannabinol (THC) Concentrations in Blood and Oral Fluid from Electronic Dance Music (EDM) Festival Attendees*. Submitted for a poster presentation at the Young Forensic Scientists Forum at the American Academy of Forensic Sciences (AAFS) Annual Meeting, Las Vegas, Nevada, February 2017.

Friscia, M., Mohr, A.L.A., Diamond, F.X., and Logan, B.K. *The Application of High Resolution Mass Spectrometry to Forensic Toxicology.* Platform presentation at the Waters Clinical Research and Forensic Technical Symposium, Plymouth Meeting, Pennsylvania, November 2016.

Friscia, M., Mohr, A.L.A., Diamond, F.X., and Logan, B.K. *Detection of Novel Psychoactive Substances in Blood and Oral Fluid from Attendees at an Electronic Dance Music Festival*. Platform presentation at the Society of Forensic Toxicologists (SOFT) Annual Meeting, Atlanta, Georgia, October 2016.

Mohr, A.L.A., Yeakel, J.K., Friscia, M., Diamond, F.X., and Logan, B.K. *Identification of Major Metabolites in Human Blood and Urine associated with the Ingestion of Methylone and Dimethylone.* Platform presentation at the Society of Forensic Toxicologists (SOFT) Annual Meeting, Atlanta, Georgia, October 2016.

Friscia, M., Wolf, S., Mohr, A.L.A., Diamond, F.X., Yeakel, J.K. and Logan, B.K. *Identification of Major Metabolites in Human Blood and Urine associated with the Ingestion of Alpha PVP*. Platform presentation at the American Academy of Forensic Sciences (AAFS), Orlando, Florida, February 2015.

Mohr, A.L.A., Yeakel, J.K., Friscia, M., and Logan, B.K. *Recreational Drug Use Trends and Emerging Analytes Identified in Blood, Urine, and/or Oral Fluid from Attendees at an Electronic Dance Music Festival.* Poster presentation at the American Academy of Forensic Sciences (AAFS), Orlando, Florida, February 2015.

Mohr, A.L.A. *Analysis of Biological Specimens for the Presence of Novel Psychoactive Substances from Attendees at an Electronic Dance Music Festival.* Platform presentation at the NIJ Grantees Meeting at the American Academy of Forensic Sciences (AAFS), Orlando, Florida, February 2015.

Yeakel, J., Mohr, A.L.A., Logan, B., Kristofic, J., and Friscia, M. *Investigation of Unknown Designer Drugs and Metabolites in Urine Collected from EDM Attendees*. Platform presentation at the NIJ Grantees Meeting at the American Academy of Forensic Sciences (AAFS), Orlando, Florida, February 2015.

Mohr, A.L.A. *Analysis of Novel Psychoactive Substances and Metabolite Discovery in Authentic Biological Samples.* Platform presentation at the International Association of Forensic Toxicologists (TIAFT), Buenos Aries, Argentina, November 2014.

Yeakel, J.K., Mohr, M., and Logan, B.K. *Comparison of GCMS, EIA, and LC-QTOF Screening Methods for Novel Psychoactive Substances in Urine Samples.* Platform presentation at the Eastern Analytical Symposium (EAS) Annual Meeting, Somerset, New Jersey, November 2014.

Yeakel, J.K., Arntson, A.L., and Logan, B.K. *Comparison of UPLC-QTOF and GCMS for Detection of Designer Drugs in Urine Samples.* Platform presentation at the Society of Forensic Toxicologists (SOFT) Annual Meeting, Grand Rapids, Michigan, October 2014.

Yeakel, J.K., Mohr, A.L.A., and Logan, B.K. *Current Patterns of Designer Drug Use in the US Electronic Dance Music Community.* Platform presentation at the International Association for Forensic Science (IAFS) Annual Meeting, Seoul, South Korea, October 2014.

Appendix A

Survey Questionnaire

Unique Identifier:

Gender:

Age:

Have you taken any medication or recreational drug in the past week? ☐ Yes ☐ No

If so, what substances?

_____

_____

_____

What symptom(s) did you experience while taking this substance(s)?

_____

_____

_____

What was your method of ingestion?

_____

_____

_____

How much did you take?

_____

_____

_____

How long ago did you take the substance?

_____

_____

Table of Contents: Appendix B-Methods

Appendix B

Amphetamines Panel in Blood 8600B

Analytes included in panel and reporting limit:

Analyte	Blood Reporting Limit
Amphetamine	5.0 ng/mL
Methamphetamine	5.0 ng/mL
Methylenedioxyamphetamine (MDA)	5.0 ng/mL
Methylenedioxyethylamphetamine (MDEA)	10 ng/mL
Methylenedioxymethamphetamine (MDMA)	5.0 ng/mL
Phendimetrazine	10 ng/mL
Phenmetrazine	5.0 ng/mL
Phentermine	10 ng/mL
Phenylpropanolamine	5.0 ng/mL
Ephedrine	5.0 ng/mL
Methylephedrine	5.0 ng/mL
Pseudoephedrine	5.0 ng/mL
Norpseudoephedrine	5.0 ng/mL
Selegiline	5.0 ng/mL

INSTRUMENT PARAMETERS:

1. **Instrumentation:** Waters Micromass Quattro Premier Tandem Mass Spectrometer with a Waters Acquity Ultra Performance LC.
2. **Column:** Acquity UPLC ™ HSS T3, 2.1 x 50 mm, 1.8 micron, part number 186003538, or equivalent
3. **Precolumn:** VanGuard Guard Cartridge, part number 186003976, or frit, part number 289002078.
4. **Mobile Phases:**
   a. A1: LC-MS Mobile Phase A, 0.1% Formic Acid in Deionized Water
   b. B1: LC-MS Mobile Phase B, 0.1% Formic Acid in Methanol
   c. Weak Wash: LC-MS Mobile Phase A, 0.1% Formic Acid in Deionized Water
   d. Strong Wash: Methanol (CH_3OH), HPLC Grade

PROCEDURE:

Calibration Curve:

Amphetamine Panel *Working* Standards

Transfer the specified amount of blank serum as outlined by the table below to appropriately labeled 12 x 75 mm test tubes. Add Amphetamine Panel Mixed Substock Standard A or B as outlined by the table below; vortex thoroughly to mix. Aliquot 0.2 mL of Standards 1-7 to appropriately labeled 12 x 75 mm test tubes and extract.

Standard	1	2	3	4	5	6	7
Aliquot 200 mcL into extraction tube.	40 mcL A + 360 mcL serum	20 mcL A + 380 mcL serum	8.0 mcL A + 392 mcL serum	20 mcL B + 380 mcL serum	8.0 mcL B + 392 mcL serum	4.0 mcL B + 396 mcL serum	2.0 mcL B + 398 mcL serum
Analyte	CONCENTRATION (ng/mL)						
Ephedrine Methylephedrine Pseudoephedrine Phenylpropanolamine Norpseudoephedrine Amphetamine Phentermine Methamphetamine MDA MDMA Phendimetrazine Phenmetrazine	1000	500	200	50	20	10	5.0
MDEA	2000	1000	400	100	40	20	10
Selegiline	500	250	100	25	10	5.0	2.5

Control:

Amphetamine Panel Low Control

Analytes	Final Concentration	Analytes	Final Concentration
Ephedrine	30 ng/mL	Methamphetamine	30 ng/mL
Methylephedrine	30 ng/mL	MDA	30 ng/mL
Pseudoephedrine	30 ng/mL	MDMA	30 ng/mL
Phenylpropanolamine	30 ng/mL	MDEA	60 ng/mL
Norpseudoephedrine	30 ng/mL	Selegiline	15 ng/mL
Amphetamine	30 ng/mL	Phendimetrazine	30 ng/mL
Phentermine	30 ng/mL	Phenmetrazine	30 ng/mL

Appendix B

Amphetamine Panel Mid Control

Analytes	Final Concentration	Analytes	Final Concentration
Ephedrine	375 ng/mL	Methamphetamine	375 ng/mL
Methylephedrine	375 ng/mL	MDA	375 ng/mL
Pseudoephedrine	375 ng/mL	MDMA	375 ng/mL
Phenylpropanolamine	375 ng/mL	MDEA	750 ng/mL
Norpseudoephedrine	375 ng/mL	Selegiline	187.5 ng/mL
Amphetamine	375 ng/mL	Phendimetrazine	375 ng/mL
Phentermine	375 ng/mL	Phenmetrazine	375 ng/mL

Amphetamine Panel High Control

Analytes	Final Concentration	Analytes	Final Concentration
Ephedrine	750 ng/mL	Methamphetamine	750 ng/mL
Methylephedrine	750 ng/mL	MDA	750 ng/mL
Pseudoephedrine	750 ng/mL	MDMA	750 ng/mL
Phenylpropanolamine	750 ng/mL	MDEA	1500 ng/mL
Norpseudoephedrine	750 ng/mL	Selegiline	375 ng/mL
Amphetamine	750 ng/mL	Phendimetrazine	750 ng/mL
Phentermine	750 ng/mL	Phenmetrazine	750 ng/mL

Samples:

1. Transfer 0.2 mL blank serum (QAS), standards, controls, and patient specimens to appropriately labeled 12 x 75 mm test tubes.
2. Add 100 mcL Amphetamine Panel *Working* Internal Standard to each test tube; vortex briefly to mix.
3. Add 200 mcL 10% Trichloroacetic Acid (TCA); vortex for ~30 seconds.
4. Centrifuge all test tubes at 3600 rpm for ~5 minutes.
5. Transfer 100 mcL of supernatant **by using a pipette (Do not pour over)** to appropriately labeled autosampler vials and cap with Teflon-lined snap-caps. Extracts are ready for LC-MS/MS analysis.

Appendix B

Analyte MRM Transitions

Analyte	Quant Ion	Ratio Ion
Ephedrine-d3	169 > 151	169 > 136
Ephedrine	166 > 148	166 > 133
Methylephedrine-d3	165 > 150	183 > 117
Methylephedrine	162 > 147	180 > 117
Pseudoephedrine-d3	169 > 151	169 > 136
Pseudoephedrine	166 > 148	166 > 133
Phenylpropanolamine-d3	155 > 137	155 > 119
Phenylpropanolamine	152 > 117	134 > 117
Norpseudoephedrine-d3	155 > 137	155 > 120
Norpseudoephedrine	152 > 117	134 > 117
Amphetamine-d5	141 > 124	141 > 93
Amphetamine	136 > 119	136 > 91
Phentermine-d5	155 > 96	155 > 138
Phentermine	150 > 91	150 > 133
Methamphetamine-d5	155 > 121	155 > 92
Methamphetamine	150 > 119	150 > 91
MDA-d5	185 > 168	185 > 110
MDA	180 > 163	180 > 135
MDMA-d5	199 > 165	199 > 135
MDMA	194 > 163	194 > 133
MDEA-d5	213 > 163	213 > 135
MDEA	208 > 163	208 > 135
Selegiline-d8	196 > 124	196 > 93
Selegiline	188 > 119	188 > 91
Phendimetrazine-d3	195 > 151	195 > 149
Phendimetrazine	192 > 148	192 > 146
Phenmetrazine-d5	183 > 122	183 > 120
Phenmetrazine	178 > 117	178 > 115

Instrumental Gradient:

Time (min)	Flow Rate	%A	%B
Initial	0.4	95	5
3.00	0.4	90	10
5.00	0.4	80	20
6.70	0.4	5	95
6.90	0.4	5	95
7.00	0.4	95	5
8.00	0.4	95	5

Appendix B

Benzodiazepines in Blood-9329B

Analytes included in panel and reporting limits:

ANALYTE	Reporting Limit	ANALYTE	Reporting Limit
Alprazolam	5.0 ng/mL	Diazepam	20 ng/mL
Hydroxyalprazolam	5.0 ng/mL	Lorazepam	5.0 ng/mL
Triazolam	2.0 ng/mL	Clonazepam	2.0 ng/mL
Hydroxytriazolam	5.0 ng/mL	7-Aminoclonazepam	5.0 ng/mL
Estazolam	5.0 ng/mL	Flurazepam	2.0 ng/mL
Midazolam	5.0 ng/mL	Hydroxyethylflurazepam	5.0 ng/mL
Nordiazepam	20 ng/mL	Desalkylflurazepam	5.0 ng/mL
Oxazepam	20 ng/mL	Chlordiazepoxide	20 ng/mL
Temazepam	20 ng/mL	Clobazam	20 ng/mL

INSTRUMENT PARAMETERS:

1. **Instrumentation:** Waters Micromass Quattro Premier or TQD Tandem Mass Spectrometer with a Waters Acquity Ultra Performance LC.
2. **Column:** Waters Acquity BEH C18, 2.1 x 100 mm, particle size 1.7 micron, part number 186002352, or equivalent, with frit, part number 289002078.
3. **Mobile Phases:**
   a. A2: LC-MS Mobile Phase A, 0.1 M Ammonium Acetate Buffer, pH 9.0
   b. B1: Methanol (CH_3OH), HPLC Grade
   c. Weak Wash: LC-MS Mobile Phase A, 0.1% Formic Acid in Deionized Water
   d. Strong Wash: 10% Ammonium Hydroxide (NH_4OH) in Methanol

PROCEDURE

Calibration Curve:

Benzodiazepine Panel Working Standards

Transfer the specified amount of blank serum (QAS) as outlined by the table below to appropriately labeled 12 x 75 mm test tubes. Add Benzodiazepine Serum Panel Substock Standard B or A as outlined by the table below; vortex thoroughly to mix. Aliquot 0.2 mL of Standards 7-1 to appropriately labeled 12 x 75 mm test tubes and extract.

Standard	7	6	5	4	3	2	1
Amount to Add	2.0 mcL B + 400 mcL Serum	4.0 mcL B + 400 mcL Serum	10 mcL B + 390 mcL Serum	20 mcL B + 380 mcL Serum	4.0 mcL A + 400 mcL Serum	10 mcL A + 390 mcL Serum	20 mcL A + 380 mcL Serum
ANALYTE	Concentrations						
Diazepam	20 ng/mL	40 ng/mL	100 ng/mL	200 ng/mL	400 ng/mL	1000 ng/mL	2000 ng/mL
Nordiazepam							
Oxazepam							
Temazepam							
Clobazam							
Chlordiazepoxide							
Lorazepam	5.0 ng/mL	10 ng/mL	25 ng/mL	50 ng/mL	100 ng/mL	250 ng/mL	500 ng/mL
7-Aminoclonazepam							
Alprazolam							
Hydroxyalprazolam							
Midazolam							
Hydroxytriazolam							
Hydroxyethylflurazepam							
Desalkylflurazepam							
Estazolam							
Clonazepam	2.0 ng/mL	4.0 ng/mL	10 ng/mL	20 ng/mL	40 ng/mL	100 ng/mL	200 ng/mL
Triazolam							
Flurazepam							

Controls Preparation:

Control	Amount to Add	Final Concentration
Low	6.0 mcL of Substock B + 394 blank serum	See Below
Mid	6.0 mcL of Substock A + 394 blank serum	See Below
High	16 mcL of Substock A + 384 blank serum	See Below

Appendix B

Control Concentrations:

Analyte	Low Concentration	Mid Concentration	High Concentration
Diazepam	60 ng/mL	600 ng/mL	1600 ng/mL
Nordiazepam	60 ng/mL	600 ng/mL	1600 ng/mL
Oxazepam	60 ng/mL	600 ng/mL	1600 ng/mL
Temazepam	60 ng/mL	600 ng/mL	1600 ng/mL
Clobazam	60 ng/mL	600 ng/mL	1600 ng/mL
Chlordiazepoxide	60 ng/mL	600 ng/mL	1600 ng/mL
Lorazepam	15 ng/mL	150 ng/mL	400 ng/mL
7-Aminoclonazepam	15 ng/mL	150 ng/mL	400 ng/mL
Alprazolam	15 ng/mL	150 ng/mL	400 ng/mL
Hydroxyalprazolam	15 ng/mL	150 ng/mL	400 ng/mL
Midazolam	15 ng/mL	150 ng/mL	400 ng/mL
Hydroxytriazolam	15 ng/mL	150 ng/mL	400 ng/mL
Hydroxyethylflurazepam	15 ng/mL	150 ng/mL	400 ng/mL
Desalkylflurazepam	15 ng/mL	150 ng/mL	400 ng/mL
Estazolam	15 ng/mL	150 ng/mL	400 ng/mL
Clonazepam	6.0 ng/mL	60 ng/mL	160 ng/mL
Triazolam	6.0 ng/mL	60 ng/mL	160 ng/mL
Flurazepam	6.0 ng/mL	60 ng/mL	160 ng/mL

Samples

1. Transfer 0.2 mL blank serum (QAS), standards, controls, and patient specimens to appropriately labeled 12 x 75 mm test tubes.
2. Add 50 mcL Benzodiazepine Panel *Working* Internal Standard to each test tube; vortex briefly to mix.
3. Add 200 mcL of Carbonate Buffer, pH 9.0; vortex for ~30 seconds.
4. Add 1.2 mL of MTBE (Methyl-t-Butyl Ether).
5. Vortex test tubes for ~1 minute on multi-vortexor at a setting of ~70 without pulsing.
6. Centrifuge all test tubes at 3600 rpm for ~5 minutes.
7. Transfer top MTBE layer to a second set of appropriately labeled 12 x 75 mm test tubes. This can be accomplished either by freezing the test tube in a dry ice/acetone bath and pouring over; or pipetting the top layer.
8. Evaporate to dryness at 40±5°C using the TurboVap for ~3-5 minutes. **DO NOT OVER-DRY.**
9. Reconstitute by adding 150 mcL of Methanol; vortex thoroughly to mix.
10. Transfer to appropriately labeled autosampler vials and cap with Teflon-lined snap-caps. Extracts are ready for LC-MS/MS analysis.

Appendix B

Analyte MRM Transitions

Analyte	Quant Ion	Ratio Ion
Diazepam-D5	290 > 154	290 > 198
Diazepam	285 > 154	285 > 193
Nordiazepam-D5	276 > 140	276 > 213
Nordiazepam	271 > 140	271 > 165
Oxazepam-D5	292 > 246	292 > 274
Oxazepam	287 > 241	287 > 269
Temazepam-D5	306 > 260	306 > 288
Temazepam	301 > 255	301 > 283
Clobazam-D5	306 > 229	306 > 264
Clobazam	301 > 224	301 > 259
Chlordiazepoxide-D5	305 > 286	305 > 232
Chlordiazepoxide	300 > 282	300 > 227
Lorazepam-D4	327 > 281	327 > 309
Lorazepam	321 > 275	321 > 303
Clonazepam-D4	320 > 274	320 > 218
Clonazepam	316 > 270	318 > 272
7-Aminoclonazepam-D4	290 > 121	290 > 226
7-Aminoclonazepam	286 > 121	286 > 222
Alprazolam-D5	314 > 286	314 > 210
Alprazolam	309 > 281	309 > 205
Hydroxyalprazolam-D5	330 > 302	330 > 284
Hydroxyalprazolam	325 > 297	325 > 279
Midazolam-D4	330 > 295	330 > 248
Midazolam	326 > 291	326 > 244
Flurazepam-D10	398 > 315	398 > 289
Flurazepam	388 > 315	388 > 288
Triazolam-D4	349 > 321	349 > 312
Triazolam	343 > 308	343 > 239
Hydroxytriazolam-D4	365 > 337	365 > 176
Hydroxytriazolam	359 > 331	359 > 176
Hydroxyethylflurazepam-D4	337 > 113	337 > 309
Hydroxyethylflurazepam	333 > 109	333 > 315
Desalkylflurazepam-D4	293 > 140	293 > 230
Desalkylflurazepam	289 > 140	289 > 226
Estazolam-D5	300 > 210	300 > 272
Estazolam	295 > 205	295 > 267

Appendix B

Instrumental Gradient

Time (min)	Flow Rate	%A	%B
Initial	0.3	80	20
1.00	0.3	57.5	42.5
1.50	0.3	45	55
4.00	0.3	45	55
6.00	0.3	15	85
6.20	0.3	10	90
6.40	0.3	80	20
7.00	0.3	80	20

Appendix B

Cocaine and Metabolites in Blood-1300B

Analytes included in panel reporting limits:

Specimen	Cocaine	Cocaethylene	Benzoylecgonine
Blood	20 ng/mL	20 ng/mL	50 ng/mL

PROCEDURE:

Calibration:

Six (6) Level of Calibrators are prepared. Using Cocaine Mixed Spiking Standard A and B use appropriate syringes perform the following spikes into **0.5 mL Serum.**

Calibrator	Final Concentration Cocaine/Cocaethylene/BZE	Amount to Spike of Stock A Mixed Standard	Amount to Spike of Stock B Mixed Standard
Level 1	2000/2000/5000 ng/mL	50.0 µL	
Level 2	500/500/1250 ng/mL	12.5 µL	
Level 3	200/200/500 ng/mL	5.0 µL	
Level 4	60/60/150 ng/mL		15.0 µL
Level 5	20/20/50 ng/mL		5.0 µL

Controls:

Control	Cocaine*	Cocaethylene*
Low Control	100 ng/mL	100 ng/mL
High Control	500 ng/mL	500 ng/mL

***Cocaine and Cocaethylene control purchased from UTAK**

Samples:

1. Carefully add 25 µL of Mixed Cocaine Internal Standard Solution (4/10/4 ng/µL) to each tube, using a properly primed repeater.
2. Add 1.0 mL of deionized water, 1.5 mL of pH 6.0-phosphate buffer (0.1M) to each tube. Vortex to mix.
3. Centrifuge at 3000 rpm for 5 minutes, while centrifuging start conditioning the columns as described in step #6.
4. Conditioning the appropriate number of *UCT* 130 mg columns for use on the manifold:
    a. 3.0 mL of Methanol
    b. 3.0 mL of DiH_2O
    c. 1.0 mL of (0.1M) pH 6.0 phosphate buffer
5. Pour samples into the corresponding columns and allow samples to flow through the column at **1-2 mL/min** using positive pressure.
6. Washing columns:
    a. 3.0 mL of DiH_2O

b. 1.5 mL of (0.1M) Acetic Acid
c. 2.0 mL of Methanol
d. 2.0 mL of Methanol

7. Eluting samples:
   a. 0.2 mL of Mixed Elution Solvent-Methylene Chloride: Isopropyl Alcohol: Ammonia Hydroxide (78:20:2) (*Must wash with exactly 0.2 mL using a repeater!* Dry done bed of column after this step (Approximately 30 seconds/no more bubbling). **Switch** the waste container with appropriately labeled 12 x 75 mm test tubes in the collection rack. Apply 3.0 mL of "mixed elution solvent" to each column and pressurize as outlined above. Solvent is collected into the 12 x 75 mm tubes. ***Critical Flow Rate Step!*** The elution must flow at approximately **1 mL/min, about 1 drop per sec.** Mixed Elution Solvent must not sit in the column for any extended period of time.
8. Place the 12 x 75 mm eluent tubes in the TurboVap® and evaporate to dryness at 55ºC ±5°C under a gentle stream of nitrogen.
9. When tubes are completely dried, add 50 µL BSTFA to each tube. Vortex briefly. Transfer BSTFA into labeled autosampler vials, and cap. Place entire rack into an oven at 70ºC (±5ºC) for 30 minutes to derivatize.
10. Extracts are ready for GC/MS analysis.
11. System Suitability must first be performed by injecting a neat or a proven successful extracted lowest level calibrator to verify instrument acceptability.

INSTRUMENT PARAMETERS

Instrumentation: Agilent 5973 Gas Chromatograph Mass Spectrometer with an Agilent 6890 Gas Chromatograph, or equivalent.

Column: Varian 17MS (15m x 0.32 mm x 15 um)

Analyte	Retention Time (min)	Relative Response Time	Target	Q1	Q2
D3-Cocaine	5.945	NA	185	306	
Cocaine	5.956	1.002	182	303	272
Cocaethylene	6.175	1.039	196	317	272
D3-BZE	5.802	N/A	243	364	
BZE	5.814	1.002	240	346	361
D3-m-OH-BZE	7.052	N/A	243	452	
m-OH-BZE	7.063	1.002	240	256	449

Appendix B

Opiates Panel in Blood-8670B

Analytes included in this panel and reporting limits

Analyte	Reporting Limit
Hydromorphone	1.0 ng/mL
Oxymorphone	1.0 ng/mL
Codeine	5.0 ng/mL
Dihydrocodeine	5.0 ng/mL
Hydrocodone	5.0 ng/mL
Morphine	5.0 ng/mL
Oxycodone	5.0 ng/mL

INSTRUMENT PARAMETERS:

1. **Instrumentation:** Waters TQS Tandem Mass Spectrometer with a Waters Acquity Ultra Performance LC system.
2. **Column:** Acquity UPLC BEH C18 2.1 x 100 mm or equivalent with frit, part no. 700003776.
3. **Mobile Phases:**
    a. A2 - Mobile Phase A, pH 4.0 Ammonium Formate Buffer
    b. B1 - Mobile Phase B, High Purity Methanol
    c. Weak Wash - Mobile Phase A, 0.1 % FA in DI water
    d. Strong Wash– Methanol (CH_3OH), High Purity

PROCEDURE:

Calibration:

Total Opiate Working Standards

Prepare a spiked Calibration Curve in 12 x 75 mm glass test tubes as outlined by the instructions below. Vortex thoroughly to mix after each step. Transfer 200 mcL of each standard to its corresponding extraction tube. When running a sample with standard addition, follow the instructions outlined below for the amount to spike.

Standard	Double spike and aliquot instructions. Spike the following amounts into 500 mcL Urine Transfer 200mcL of each standard to its corresponding extraction tube.	Concentration of Hydromorphone and Oxymorphone (ng/mL)	Concentration of Codeine, Dihydrocodeine, Hydrocodone, Morphine, and Oxycodone (ng/mL)
1	50 mcL Standard A	200	1000
2	25 mcL of Standard A	100	500
3	5 mcL of Standard A	20	100
4	10 mcL of Standard B	4	20
5	5 mcL of Standard B	2	10
6	2.5 mcL of Standard B	1	5

Controls:

Analyte	Low Control	High Control
Hydromorphone	12 ng/mL	600 ng/mL
Oxymorphone	12 ng/mL	600 ng/mL
Codeine	60 ng/mL	3000 ng/mL
Dihydrocodeine	60 ng/mL	3000 ng/mL
Hydrocodone	60 ng/mL	3000 ng/mL
Morphine	60 ng/mL	3000 ng/mL
Oxycodone	60 ng/mL	3000 ng/mL

Appendix B

Samples:

1. Transfer 0.20 mL blank deionized water, standards, controls, and patient specimens to appropriately labeled 12 x 75 mm test tubes.
2. Add 25 mcL Total Opiate Working Internal Standard, 0.08, 0.4 ng/mcL to all test tubes.
3. Add 50 mcL of hydrolysis buffer to all tubes.
4. Add 40 mcL of IMCSzyme enzyme to all tubes; vortex gently and briefly. Cap tubes. Store IMCSzyme refrigerated (2°C – 10°C). Discard if enzyme solution becomes cloudy.
5. Heat at 55°C in a heat block or water bath for 60 minutes. Allow to cool to room temperature for 15 minutes.
6. Add 1.0 mL 1.0 M Acetic Acid to each test tube; vortex briefly to mix.
7. Centrifuge tubes at 3600 rpm for five minutes
8. Tubes are now ready for extraction on the positive manifold.
9. Transfer tubes to SPE Ware racks. Place an appropriately labeled 12 x 75 mm collection test tube in the racks for each sample tube. Place a corresponding number of UCT CSDAU133 columns into the SPE Ware rack for each tube to be extracted.
10. Condition the UCT CSDAU133 columns with 2.0 mL of Methanol.
11. Equilibrate the UCT CSDAU133 columns with 2.0 mL of DI Water.
12. Transfer prepared samples to UCT CSDAU133 columns; aspirate slowly through packing bed.
13. Rinse the UCT CSDAU133 columns with 2.0 mL 1.0 M Acetic Acid.
14. Rinse the UCT CSDAU133 columns with 2.0 mL Methanol.
15. Rinse the UCT CSDAU133 columns with 2.0 mL Ethyl Acetate.
16. Elute with 2.0 mL “Opiate Mixed Elution Solvent” (78 Ethyl Acetate: 2 Ammonium Hydroxide: 20 Isopropanol) into appropriately labeled12 x 75 mm collection test tubes. Make fresh daily.
17. Evaporate to dryness at 40±5ºC using the TurboVap.
18. Reconstitute with 200 mcL 95/5 Mobile Phase Mixture [95% 0.1% Formic Acid in DI water / 5% 0.1% Formic Acid in Methanol].
19. Vortex briefly and transfer to labeled auto-sampler vials. Cap with Teflon-lined pre-slit snap-caps or equivalent. Extracts are ready for LC-MS/MS analysis.

Appendix B

Analyte MRM Transitions

Analyte	Quant Ion	Ratio Ion
D3-Morphine	289.2 > 201.1	289.2 > 165.1
Morphine	286.2 > 165.1	286.2 > 201.1
D3-Oxymorphone	305.1 > 230.1	305.1 > 201.1
Oxymorphone	302.1 > 198.1	302.1 > 227.1
D3-Hydromorphone	289.2 > 185.1	289.2 > 157.1
Hydromorphone	286.2 > 185.1	286.2 > 157.1
D6-Dihydrocodeine	308.14 > 173.9	308.14 > 201.92
Dihydrocodeine	302.14 > 170.9	302.14 > 198.92
D6-Codeine	306.12 > 164.98	306.12 > 217.98
Codeine	300.12 > 164.98	300.12 > 214.98
D6-Oxycodone	322.11 > 246.92	322.11 > 261.94
Oxycodone	316.11 > 240.92	316.11 > 255.94
D6-Hydrocodone	306.05 > 173.84	306.05 > 201.92
Hydrocodone	300.05 > 170.84	300.05 > 198.92

Gradient:

Time (min)	Flow Rate	%A	%B
Initial	0.4	95	5
4.00	0.4	70	30
4.50	0.4	5	95

Appendix B

Cannabinoids Panel in Blood-0960B

Analytes included in panel and reporting limits:

Analyte	Reporting Limit
Delta-9-THC	1.0 ng/mL
Cannabidiol	1.0 ng/mL
Delta-9-Carboxy THC	5.0 ng/mL
11-Hydroxy-Delta-9-THC	5.0 ng/mL

INSTRUMENT PARAMETERS:

Instrumentation: GCxGCxGC/MS: Agilent 5975 Gas Chromatograph Mass Spectrometer with an Agilent 7890 Gas Chromatograph, or equivalent.

1. Column 1: DB5MS (5m x 0.25 x 0.25)
2. Column 2: DB17MS (15m x 0.25 x 0.25)
3. Column 3: DB1MS (15 m x 0.25 x 0.25)

Analyte	Ions Monitored			Acceptance Range
	Target	Q1	Q2	
D3-Cannabidiol	393	340	354	±20%
Cannabidiol	390	301	391	±20%
D3-THC	374	389		±20%
THC	371	303	386	±20%
D3-THCC	374	491	476	±20%
THCC	371	488	473	±20%
D3-11-OH THC	374	462	477	±20%
11-OH THC	371	459	474	±20%

Appendix B

PROCEDURE:

Calibration:

Cannabinoids Working Standards

Spike the calibration curve into 0.50 mL blank blood with the mixed Cannabinoids Standards A (1/1/5/5 ng/mcL) and B (0.1/0.1/0.5/0.5 ng/mcL) according to the following chart. **Prepare Standard B (1+9 of A) daily.**

Calibrator	Final Concentration THC/Cannabidiol/THCC/11 OH THC	Amount of Mixing Spiking Standard to spike	Calibration STD
Level 1	1/1/5/5 ng/mL	5 µL	B (1+9 of A)
Level 2	2.5/2.5/12.5/12.5 ng/mL	12.5 µL	B (1+9 of A)
Level 3	7/7/35/35 ng/mL	3.5 µL	A
Level 4	15/15/75/75 ng/mL	7.5 µL	A
Level 5	30/30/150/150 ng/mL	15 µL	A
Level 6	50/50/250/250 ng/mL	25 µL	A
Negative	---	-	

Controls:

Analyte	High QC Hand Spike Solution	High QC Target
Delta-9-THC	1.0 ng/mcL	20 ng/mL
Cannabidiol	1.0 ng/mcL	20 ng/mL
11-Hydroxy-Delta-9-THC	5.0 ng/mcL	100 ng/mL
Delta-9-Carboxy THC*	N/A	100 ng/mL

Analyte	High QC Hand Spike Solution	High QC Target
Delta-9-THC	0.15 ng/mcL	3 ng/mL
Cannabidiol	0.15 ng/mcL	3 ng/mL
11-Hydroxy-Delta-9-THC	0.75 ng/mcL	15 ng/mL
Delta-9-Carboxy THC*	N/A	15 ng/mL

***Not prepare via hand spiking**

Appendix B

Samples:

1. Using an Eppendorf pipette, transfer 0.50 mL of specimen into a labeled 13X100 mm disposable culture tube according to prep batch.
2. Carefully add 50 mcL of mixed internal standard (0.2 ng/mcL) to all tubes using the repeater. Vortex briefly.
3. Add 1.0 mL of 50 mM phosphoric acid in de-ionized water using an Oxford Pipettor. Vortex briefly.
4. Add 2.5 mL of CAN Extraction Solvent: hexane/ethyl acetate/methyl-tert-butyl-ether (80/10/10) to each tube.
5. Cap and rotate tubes for 15 minutes.
6. Centrifuge tubes for 10 minutes at 3750 rpm. Prepare a set of labeled 12X75 mm test tubes.
7. Using glass pipette tips or freeze samples in a dry ice-acetone bath to transfer the upper organic layer into labeled 12X75 mm tubes. It is important not to transfer any of the aqueous layer.
8. Transfer tubes containing organic layer to the TurboVap and dry down at ±55ºC±5ºC under a gentle (<15 psi) stream of nitrogen.
9. When tubes are completely dried, add 50 mcL BSTFA + 1% TMCS into each tube using a repeater. Cap securely, vortex, and derivatize at 70ºC±5ºC for 30 minutes.
10. Cool tubes and transfer reconstituted extracts to appropriately labeled autosampler vials with conical micro inserts. Extracts are ready for GC/MS analysis.
11. Sequence and place samples into the designated autosampler positions.

Appendix B

Oral Fluid Confirmation, Qualitative Common Drugs of Abuse Panel

Analytes included in panel and reporting limits:

Analyte	Reporting Limit	Analyte	Reporting Limit
Amphetamine	2.5 ng/mL	Morphine	2.0 ng/mL
Methamphetamine	2.5 ng/mL	Hydrocodone	2.0 ng/mL
Methylenedioxyamphetamine [MDA]	2.5 ng/mL	6-Acetylmorphine [6-MAM]	2.0 ng/mL
Methylenedioxymethamphetamine [MDMA]	2.5 ng/mL	Hydromorphone	2.0 ng/mL
Diazepam	1.5 ng/mL	Oxycodone	2.0 ng/mL
Nordiazepam	1.5 ng/mL	Oxymorphone	2.0 ng/mL
Oxazepam	2.25 ng/mL	Dihydrocodeine	2.0 ng/mL
Temazepam	1.5 ng/mL	Cocaine	2.5 ng/mL
Chlordiazepoxide	25 ng/mL	Benzoylecgonine	1.25 ng/mL
Lorazepam	1.5 ng/mL	Cocaethylene	1.25 ng/mL
Clonazepam	1.5 ng/mL	Methadone	2.5 ng/mL
Alprazolam	1.5 ng/mL	EDDP	2.5 ng/mL
Midazolam	2.25 ng/mL	Phencyclidine [PCP]	1.0 ng/mL
Codeine	2.0 ng/mL	Dextromethorphan	25 ng/mL

INSTRUMENT PARAMETERS:

1. **Instrumentation:** Waters TQD Tandem Mass Spectrometer with a Waters Acquity Ultra Performance LC system.
2. **Column:** Waters BEH C18, 2.1 x 100 mm, particle size 1.7 micron, part number 186002352, or equivalent type L1 column with frit, part number 289002078.
3. **Mobile Phases:**
   a. A2: LC-MS Mobile Phase A, Ammonium Formate, pH 4.0
   b. B2: LC-MS Mobile Phase B, 0.1% Ammonium Hydroxide in Methanol
   c. Weak Wash: LC-MS Mobile Phase A, 0.1% Formic Acid in Deionized Water
   d. Strong Wash: Methanol (CH_3OH), HPLC Grade

PROCEDURE:

Oral Fluid Confirmation Working Standard

Transfer 0.5 mL of Oral Fluid Confirmation BULK Standard to an appropriately labeled 13 x 100 mm test tube and spike 10 mcL of Mixed Handspike Substock Standard; vortex thoroughly to mix and extract.

Duplicate single-point calibrators are run in the beginning and end of the batch and both points are used to create a calibration curve that goes through zero. The reporting limits for the analytes in this panel as determined by the single-point calibrator are as follows:

Analyte	Stock Concentration	Cutoff Concentration
Amphetamine	0.125 ng/mcL	2.5 ng/mL
Methamphetamine	0.125 ng/mcL	2.5 ng/mL
MDA	0.125 ng/mcL	2.5 ng/mL
MDMA	0.125 ng/mcL	2.5 ng/mL
Diazepam	0.075 ng/mcL	1.5 ng/mL
Nordiazepam	0.075 ng/mcL	1.5 ng/mL
Oxazepam	0.1125 ng/mcL	2.25 ng/mL
Temazepam	0.075 ng/mcL	1.5 ng/mL
Chlordiazepoxide	1.25 ng/mcL	25 ng/mL
Lorazepam	0.075 ng/mcL	1.5 ng/mL
Clonazepam	0.075 ng/mcL	1.5 ng/mL
Alprazolam	0.075 ng/mcL	1.5 ng/mL
Midazolam	0.1125 ng/mcL	2.25 ng/mL
Codeine	0.1 ng/mcL	2.0 ng/mL
Morphine	0.1 ng/mcL	2.0 ng/mL
Hydrocodone	0.1 ng/mcL	2.0 ng/mL
6-MAM	0.1 ng/mcL	2.0 ng/mL
Hydromorphone	0.1 ng/mcL	2.0 ng/mL
Oxycodone	0.1 ng/mcL	2.0 ng/mL
Oxymorphone	0.1 ng/mcL	2.0 ng/mL
Dihydrocodeine	0.1 ng/mcL	2.0 ng/mL
Cocaine	0.125 ng/mcL	2.5 ng/mL
Benzoylecgonine	0.0625 ng/mcL	1.25 ng/mL
Cocaethylene	0.0625 ng/mcL	1.25 ng/mL
Methadone	0.125 ng/mcL	2.5 ng/mL

Appendix B

EDDP	0.125 ng/mcL	2.5 ng/mL
PCP	0.05 ng/mcL	1.0 ng/mL
Dextromethorphan	1.25 ng/mcL	25 ng/mL

Positive Control Preparation Instructions

Transfer 0.5 mL of Oral Fluid Confirmation Positive Control (125% Cutoff Concentration) to an appropriately labeled 13 x 100 mm test tube and spike 12.5 mcL of Mixed Handspike QC Substock; vortex thoroughly to mix and extract. The QC values for the analytes in this panel are as follows:

Analyte	Stock Concentration	Cutoff Concentration	Positive Control Concentration
Amphetamine	0.125 ng/mcL	2.5 ng/mL	3.125 ng/mL
Methamphetamine	0.125 ng/mcL	2.5 ng/mL	3.125 ng/mL
MDA	0.125 ng/mcL	2.5 ng/mL	3.125 ng/mL
MDMA	0.125 ng/mcL	2.5 ng/mL	3.125 ng/mL
Diazepam	0.075 ng/mcL	1.5 ng/mL	1.875 ng/mL
Nordiazepam	0.075 ng/mcL	1.5 ng/mL	1.875 ng/mL
Oxazepam	0.1125 ng/mcL	2.25 ng/mL	2.8125 ng/mL
Temazepam	0.075 ng/mcL	1.5 ng/mL	1.875 ng/mL
Chlordiazepoxide	1.25 ng/mcL	25 ng/mL	31.25 ng/mL
Lorazepam	0.075 ng/mcL	1.5 ng/mL	1.875 ng/mL
Clonazepam	0.075 ng/mcL	1.5 ng/mL	1.875 ng/mL
Alprazolam	0.075 ng/mcL	1.5 ng/mL	1.875 ng/mL
Midazolam	0.1125 ng/mcL	2.25 ng/mL	2.8125 ng/mL
Codeine	0.1 ng/mcL	2.0 ng/mL	2.5 ng/mL
Morphine	0.1 ng/mcL	2.0 ng/mL	2.5 ng/mL
Hydrocodone	0.1 ng/mcL	2.0 ng/mL	2.5 ng/mL
6-MAM	0.1 ng/mcL	2.0 ng/mL	2.5 ng/mL
Hydromorphone	0.1 ng/mcL	2.0 ng/mL	2.5 ng/mL
Oxycodone	0.1 ng/mcL	2.0 ng/mL	2.5 ng/mL
Oxymorphone	0.1 ng/mcL	2.0 ng/mL	2.5 ng/mL
Dihydrocodeine	0.1 ng/mcL	2.0 ng/mL	2.5 ng/mL
Cocaine	0.125 ng/mcL	2.5 ng/mL	3.125 ng/mL
Benzoylecgonine	0.0625 ng/mcL	1.25 ng/mL	1.5625 ng/mL
Cocaethylene	0.0625 ng/mcL	1.25 ng/mL	1.5625 ng/mL

Methadone	0.125 ng/mcL	2.5 ng/mL	3.125 ng/mL
EDDP	0.125 ng/mcL	2.5 ng/mL	3.125 ng/mL
PCP	0.05 ng/mcL	1.0 ng/mL	1.25 ng/mL
Dextromethorphan	1.25 ng/mcL	25 ng/mL	31.25 ng/mL

PROCEDURE:

***CAUTION*:** During this procedure you will be working with potentially infectious materials (oral fluid) and potentially hazardous chemicals. You must follow the safety procedures for handling these materials as detailed in NMS Labs' Bloodborne Pathogen Exposure Control Plan and Chemical Hygiene Plan.

1. Transfer 0.50 mL negative synthetic saliva, standards, controls, and patient specimens to appropriately labeled 13 x 100 mm test tubes.
   Oral Fluid Confirmation *Working* Calibrators and Controls
   Transfer the following amounts as outlined by the table below to an appropriately labeled 13 x 100 mm test tube; vortex thoroughly to mix. Duplicate single-point calibrators are run at the beginning and end of the batch and both points are used to create a calibration curve that goes through zero.

Calibrator/Control	Amount to Aliquot of Bulk		Amount to Spike of Mixed Substock
Cut-off Calibrator (100% Standard)	0.5 mL Bulk Standard	**and**	10 mcL Mixed Substock Standard
Positive Control (125% Reporting Limit)	0.5 mL Bulk Control	**and**	12.5 mcL Mixed Handspike QC Substock

Standard Addition Preparation Instructions:
Transfer 10 mcL Mixed Handspike Substock Standard to 0.5 mL of sample and add 0.5 mL of Oral Fluid Confirmation BULK Standard. Add 25 mcL of Oral Fluid Confirmation *Working* Internal Standard, 1.0 ng/mcL, 1.5 mL 1.0 M Acetic Acid (from Procedure Step 6), and continue procedure as normal.

2. Add 25 mcL Oral Fluid Confirmation *Working* Internal Standard, 1.0 ng/mcL, to all test tubes; vortex briefly to mix.
3. Place a corresponding number of polymer Strata-X-C columns (60 mg/3 mL part number 8B-S029-UBL) into the SPE Ware rack for each sample to be extracted.
4. Condition the polymer Strata-X-C columns with 2.0 mL Methanol.
5. Condition the polymer Strata-X-C columns with 2.0 mL Deionized Water.

6. **After** conditioning the extraction columns, add 2.0 mL 1.0 M Acetic Acid to each test tube; vortex briefly to mix.
7. Samples are now ready for extraction on the positive manifold.
8. Transfer prepared samples to the polymer Strata-X-C columns using a Pasteur pipette; aspirate slowly through packing bed.
9. Rinse the polymer Strata-X-C columns with 2.0 mL 1.0 M Acetic Acid.
10. Rinse the polymer Strata-X-C columns with 2.0 mL "Mixed Solvent" (60% 1.0 M Acetic Acid / 40% Methanol). Dry extraction columns at full flow for ~ 30 seconds.
11. Rinse the polymer Strata-X-C columns with 2.0 mL Hexane.
12. Add 50 mcL Methanolic HCl (1%) to empty 12 x 75 mm test tubes before elution for each sample tube. Transfer test tubes to SPE Ware rack.
13. Elute with 2.0 mL 5% Ammonium Hydroxide in Methanol into appropriately labeled12 x 75 mm collection test tubes containing methanolic HCl (1%).
14. Evaporate to dryness at 40±5ºC using the TurboVap **set at 5 psi or less** (pressure >5 psi can cause contamination) for ~25 minutes.
15. Reconstitute with 200 mcL 80:20 Deionized Water / Methanol; vortex thoroughly to mix.
16. Transfer to appropriately labeled autosampler vials and cap with Teflon-lined snap-caps or equivalent. Extracts are ready for LC-MS/MS analysis.

Analyte MRM Transitions

Analyte	Quant Ion	Ratio Ion
Morphine	286.2 > 165.0	286.2 > 153.0
Morphine-D3	289.2 > 165.0	289.2 > 153.0
Oxymorphone	302.2 > 197.9	302.2 > 226.9
Oxymorphone-D3	305.2 > 201.0	305.2 > 230.0
Hydromorphone	286.2 > 185.2	286.2 > 157.1
Hydromorphone-D3	289.2 > 185.2	289.2 > 157.1
Dihydrocodeine	302.2 > 199.0	302.2 > 171.0
Dihydrocodeine-D6	308.2 > 202.0	308.2 > 174.0
Codeine	300.2 > 58.2	300.2 > 165.1
Codeine-D6	306.2 > 61.2	306.2 > 165.1
Oxycodone	316.2 > 241.2	316.2 > 256.2
Oxycodone-D6	322.2 > 247.2	322.2 > 262.2
Hydrocodone	300.2 > 199.1	300.2 > 171.1
Hydrocodone-D6	306.2 > 202.2	306.2 > 174.1
6-MAM	328.2 > 165.1	328.2 > 211.1
6-MAM-D6	334.2 > 165.1	334.2 > 211.1
Amphetamine	136.1 > 119.0	136.1 > 91.0
Amphetamine-D5	141.1 > 124.1	141.1 > 93.0

Appendix B

MDA	180.1 > 135.0	180.1 > 163.0
MDA-D5	185.1 > 110.0	185.1 > 168.1
Methamphetamine	150.1 > 91.0	150.1 > 119.0
Methamphetamine-D5	155.1 > 121.1	155.1 > 92.0
MDMA	194.1 > 133.0	194.1 > 163.0
MDMA-D5	199.1 > 135.1	199.1 > 165.1
Benzoylecgonine	290.2 > 168.0	290.2 > 105.0
Benzoylecgonine-D3	293.2 > 171.0	293.2 > 105.0
Cocaine	304.2 > 182.0	304.2 > 82.0
Cocaine-D3	307.2 > 185.1	307.2 > 85.0
Cocaethylene	318.2 > 196.2	318.2 > 81.9
Cocaethylene-D3	321.2 > 199.2	321.2 > 84.9
Phencyclidine	244.2 > 86.0	244.2 > 159.1
Phencyclidine-D5	249.2 > 86.0	249.2 > 164.1
EDDP	278.2 > 234.1	278.2 > 249.2
EDDP-D3	281.2 > 234.1	281.2 > 249.2
Dextromethorphan	272.2 > 147.0	272.2 > 215.2
Dextromethorphan-D3	275.2 > 147.0	275.2 > 215.2
Clonazepam	316.0 > 270.0	316.0 > 214.0
Clonazepam-D4	320.0 > 274.0	320.0 > 218.0
Oxazepam	287.0 > 241.0	289.0 > 243.0
Oxazepam-D5	292.0 > 246.0	294.0 > 248.0
Lorazepam	321.0 > 275.0	323.0 > 277.0
Lorazepam-D4	325.0 > 279.0	327.0 > 281.0
Alprazolam	309.0 > 205.0	311.0 > 205.0
Alprazolam-D5	314.0 > 210.0	316.0 > 210.0
Methadone	310.2 > 265.2	310.2 > 105.0
Methadone-D9	319.2 > 268.2	319.2 > 105.0
Temazepam	301.0 > 255.0	303.0 > 257.0
Temazepam-D5	306.0 > 260.0	308.0 > 262.0
Chlordiazepoxide	300.0 > 227.0	300.0 > 282.0
Chlordiazepoxide-D5	305.0 > 232.0	305.0 > 286.0
Nordiazepam	271.0 > 140.0	271.0 > 165.0
Nordiazepam-D5	276.0 > 140.0	276.0 > 165.0
Midazolam	326.0 > 291.0	326.0 > 244.0
Midazolam-D4	330.0 > 295.0	330.0 > 248.0
Diazepam	285.0 > 154.0	285.0 > 193.0
Diazepam-D5	290.0 > 154.0	290.0 > 198.0

Appendix B

Gradient

Time (min)	Flow Rate	%A	%B
Initial	0.4	95	5
4.00	0.4	70	30
4.10	0.4	55	45
8.00	0.4	30	70
8.50	0.4	5	95

Appendix B

NPS Panel in Blood

Analytes included in panel and reporting limit:

Analyte	Reporting Limit
Methylone	5 ng/mL
Dimethylone	5 ng/mL
Ethylone	5 ng/mL
Butylone	5 ng/mL
Alpha-PVP	5 ng/mL
4-Fluoroamphetamine	5 ng/mL

INSTRUMENT PARAMETERS:

1. **Instrumentation:** Waters Quattro Micro Tandem Mass Spectrometer with a Waters Acquity Ultra Performance LC system.
2. **Column:** Waters BEH C18, 2.1 x 50 mm, particle size 1.7 micron
3. **Mobile Phases:**
   a. A2: LC-MS Mobile Phase A, 0.1% Formic Acid in Deionized Water
   b. B2: LC-MS Mobile Phase B, 0.1% Formic Acid in Methanol
   c. Weak Wash: LC-MS Mobile Phase A, 0.1% Formic Acid in Deionized Water
   d. Strong Wash: Methanol (CH_3OH), HPLC Grade

PROCEDURE

Calibration:

Oral Fluid Confirmation *Working* Calibrators and Controls

Transfer the following amounts as outlined by the table below to an appropriately labeled 13 x 100 mm test tube that contains 0.5 mL of blood; vortex thoroughly to mix.

Standard	Spiking Standard	Spike	Final Concentration
1	Standard B (5 ng/uL)	50 uL	500 ng/mL
2	Standard B (5 ng/uL)	25 uL	250 ng/mL
3	Standard B (5 ng/uL)	10 uL	100 ng/mL
4	Standard B (5 ng/uL)	5 uL	50 ng/mL
5	Standard C (0.5 ng/uL)	10 uL	10 ng/mL
6	Standard C (0.5 ng/uL)	5 uL	5 ng/mL

Appendix B

Controls:

QC	Spike	Sheep's Blood (uL)	Final Concentration
High Pos	35 uL QC A (5 ng/uL)	500	350 ng/mL
Low Pos	15 mcL QC Low (0.5 ng/uL)	500	15 ng/mL

Samples:

1. Transfer 0.50 mL blank deionized water, standards, controls, and patient specimens to appropriately labeled 13 x 100 mm test tubes.
2. Add 100 mcL Designer Panel *Working* Internal Standard to each test tube; vortex briefly to mix.
3. Add 1 mL of Borax Buffer, pH 10.4; vortex for ~30 seconds.
4. Add 3 mL of 70:30 N-butyl chloride:Ethyle acetate
5. Cap and rotate all tubes for 5-10 minutes.
6. Centrifuge all test tubes at 3750 rpm for ~5 minutes.
7. Transfer top 70:30 n-butyl chloride:ethyl acetate layer to a second set of appropriately labeled 13 x 100 mm test tubes. This can be accomplished either by freezing the test tube in a dry ice/acetone bath and pouring over; or pipetting the top layer.
8. Add 100 uL of a 10% HCl solution to all tubes.
9. Evaporate to dryness at 35±5°C using the TurboVap for ~3-5 minutes. **DO NOT OVER-DRY.**
10. Reconstitute by adding 200 mcL of 85:15 Mobile phase A:Mobile phase B; vortex thoroughly to mix.
11. Transfer to appropriately labeled autosampler vials and cap with Teflon-lined screw-top split top caps. Extracts are ready for LC-MS/MS analysis.

Analyte MRM Transitions

Analyte	Quant Ion	Ratio Ion
Methylone	208.2>160.1	208.2>132.1
Dimethylone	222.3>71.8	222.3>147
Ethylone	222.1>174.1	222.2>204.1
Butylone	222.1>191.1	222.1>174.1
Alpha-PVP	232.2>126.1	232.2>161.1
4-Fluoroamphetamine	154.1>109	154.1>82.8
Methylone D3	211.2>163.1	211.2>193.1
Alpha PVP D8	240.2>134.1	240.2>135

Appendix B

Gradient

Time (min)	Flow Rate	%A	%B
Initial	0.2	85	15
1.00	0.2	85	15
5.00	0.2	65	35
6.00	0.2	10	90
6.10	0.2	90	10
8.00	0.2	90	10

Appendix B

NPS panel in Oral Fluid

Analytes included in panel and reporting limit:

Analyte	Reporting Limit
Methylone	5 ng/mL
Dimethylone	5 ng/mL
Ethylone	5 ng/mL
Butylone	5 ng/mL
Alpha-PVP	5 ng/mL
4-Fluoroamphetamine	5 ng/mL

INSTRUMENT PARAMETERS:

1. **Instrumentation:** Waters Quattro Micro Tandem Mass Spectrometer with a Waters Acquity Ultra Performance LC system.
2. **Column:** Waters BEH C18, 2.1 x 50 mm, particle size 1.7 micron
3. **Mobile Phases:**
   a. A2: LC-MS Mobile Phase A, 0.1% Formic Acid in Deionized Water
   b. B2: LC-MS Mobile Phase B, 0.1% Formic Acid in Methanol
   c. Weak Wash: LC-MS Mobile Phase A, 0.1% Formic Acid in Deionized Water
   d. Strong Wash: Methanol (CH_3OH), HPLC Grade

PROCEDURE

Calibration:

Oral Fluid NPS Confirmation *Working* Calibrators and Controls

Transfer the following amounts as outlined by the table below to an appropriately labeled 13 x 100 mm test tube that contains 1 mL of oral fluid; vortex thoroughly to mix. Transfer that 1 mL of spiked oral fluid (spiked following chart below) to 3 mL of quantisal collection buffer, sample 0.5 mL of the Quantisal/sample mixture and add that to a new labeled 13X100 test tube. Continue to "Samples" instructions.

Standard	Spiking Solution	Spike	Final Concentration
1	Standard B (50 ng/uL)	40 uL	2000 ng/mL
2	Standard B (50 ng/uL)	20 uL	1000 ng/mL
3	Standard B (50 ng/uL)	15 uL	750 ng/mL
4	Standard B (5 ng/uL)	80 uL	400 ng/mL
5	Standard C (5 ng/uL)	40 uL	200 ng/mL
6	Standard C (0.5 ng/uL)	80 uL	40 ng/mL

Appendix B

Controls:

QC	Spike	Sheep's Blood (uL)	Final Concentration
High Pos	35 uL QC A (5 ng/uL)	500	350 ng/mL
Low Pos	15 mcL QC Low (0.5 ng/uL)	500	15 ng/mL

Samples:

1. Transfer 0.50 mL blank deionized water, standards, controls, and patient specimens to appropriately labeled 13 x 100 mm test tubes.
2. Add 100 mcL Designer Panel *Working* Internal Standard to each test tube; vortex briefly to mix.
3. Add 1 mL of Borax Buffer, pH 10.4; vortex for ~30 seconds.
4. Add 3 mL of 70:30 N-butyl chloride:Ethyle acetate
5. Cap and rotate all tubes for 5-10 minutes.
6. Centrifuge all test tubes at 3750 rpm for ~5 minutes.
7. Transfer top 70:30 n-butyl chloride:ethyl acetate layer to a second set of appropriately labeled 13 x 100 mm test tubes. This can be accomplished either by freezing the test tube in a dry ice/acetone bath and pouring over; or pipetting the top layer.
8. Add 100 uL of a 10% HCl solution to all tubes.
9. Evaporate to dryness at 35±5°C using the TurboVap for ~3-5 minutes. **DO NOT OVER-DRY.**
10. Reconstitute by adding 200 mcL of 85:15 Mobile phase A:Mobile phase B; vortex thoroughly to mix.
11. Transfer to appropriately labeled autosampler vials and cap with Teflon-lined screw-top split top caps. Extracts are ready for LC-MS/MS analysis.

Analyte MRM Transitions

Analyte	Quant Ion	Ratio Ion
Methylone	208.2>160.1	208.2>132.1
Dimethylone	222.3>71.8	222.3>147
Ethylone	222.1>174.1	222.2>204.1
Butylone	222.1>191.1	222.1>174.1
Alpha-PVP	232.2>126.1	232.2>161.1
4-Fluoroamphetamine	154.1>109	154.1>82.8
Methylone D3	211.2>163.1	211.2>193.1
Alpha PVP D8	240.2>134.1	240.2>135

Appendix B

Gradient

Time (min)	Flow Rate	%A	%B
Initial	0.2	85	15
1.00	0.2	85	15
5.00	0.2	65	35
6.00	0.2	10	90
6.10	0.2	90	10
8.00	0.2	90	10

Appendix B

Synthetic Cannabinoids in Oral Fluid

Analytes included in panel reporting limits

Analyte	Reporting Limit	Analyte	Reporting Limit
JWH-018	0.1 ng/mL	AB-001	0.1 ng/mL
AM-2201	0.1 ng/mL	5F-APICA	1.0 ng/mL
JWH-122	0.1 ng/mL	APICA	0.2 ng/mL
JWH-210	0.2 ng/mL	PB-22	0.1 ng/mL
JWH-081	0.1 ng/mL	APINACA	1.0 ng/mL
UR-144	0.2 ng/mL	5F-PB-22	0.1 ng/mL
XLR-11	0.2 ng/mL	5F-APINACA	1.0 ng/mL
AB-FUBINACA	1.0 ng/mL	BB-22	0.1 ng/mL
ADBICA	1.0 ng/mL	FUBIMINA	0.1 ng/mL
5F-ADBICA	1.0 ng/mL	THJ-2201	0.1 ng/mL
ADB-PINACA	0.2 ng/mL	THJ-018	0.1 ng/mL
ADB-FUBINACA	1.0 ng/mL	5F-AB-001	1.0 ng/mL
5F-ADB-PINACA	1.0 ng/mL	AB-PINACA	0.2 ng/mL
AB-CHMINACA	1.0 ng/mL		

INSTRUMENT PARAMETERS:

1. Instrumentation - Waters Premier or TQD Mass Spectrometer with an ACQUITY UPLC and LaserJet printer.
2. Column – ACQUITY BEH C18 100x2.1mm 1.7 micron column with frit.
3. Precolumn: ACQUITY UPLC BEH C18 VanGuard Pre-column 2.1x5mm, 1.7 micron, part number 186003975.
4. Mobile phase: LC-MS Mobile Phase
   a. A1: 0.1%FormicAcid in LCMS grade water
   b. B2: Acetonitrile, LCMS grade
   c. Weak Wash: 0.1% Formic Acid in DI water
   d. Strong Wash: Methanol

PROCEDURE:

Synthetic Cannabinoids in Oral Fluid *working* Controls

Transfer 50 mcL of Synthetic Cannabinoids in Oral Fluid 50% and 150% Cut-off QC stock to separate 13x100mm glass test tubes containing 450 mcL synthetic oral fluid; vortex to mix.

Analyte	50% Cut off QC	150% Cut off QC
JWH-018	0.05 ng/mL	0.15 ng/mL
AM-2201	0.05 ng/mL	0.15 ng/mL
JWH-122	0.05 ng/mL	0.15 ng/mL

JWH-210	0.1 ng/mL	0.3 ng/mL
JWH-081	0.05 ng/mL	0.15 ng/mL
UR-144	0.1 ng/mL	0.3 ng/mL
XLR-11	0.1 ng/mL	0.3 ng/mL
AB-FUBINACA	0.5 ng/mL	1.5 ng/mL
ADBICA	0.5 ng/mL	1.5 ng/mL
5F-ADBICA	0.5 ng/mL	1.5 ng/mL
ADB-PINACA	0.1 ng/mL	0.3 ng/mL
ADB-FUBINACA	0.5 ng/mL	1.5 ng/mL
5F-ADB-PINACA	0.5 ng/mL	1.5 ng/mL
AB-001	0.05 ng/mL	0.15 ng/mL
F5-APICA	0.5 ng/mL	1.5 ng/mL
APICA	0.1 ng/mL	0.3 ng/mL
PB-22	0.05 ng/mL	0.15 ng/mL
APINACA	0.5 ng/mL	1.5 ng/mL
5F-PB-22	0.05 ng/mL	0.15 ng/mL
5F-APINACA	0.5 ng/mL	1.5 ng/mL
BB-22	0.05 ng/mL	0.15 ng/mL
FUBIMINA	0.05 ng/mL	0.15 ng/mL
THJ-2201	0.05 ng/mL	0.15 ng/mL
THJ-018	0.05 ng/mL	0.15 ng/mL
5F-AB-001	0.5 ng/mL	1.5 ng/mL
AB-PINACA	0.1 ng/mL	0.3 ng/mL
AB-CHMINACA	0.5 ng/mL	1.5 ng/mL

Samples:

Note: Maximum Allowable Dilution: Oral fluid samples should not be diluted.

1. Transfer 0.5 mL blank synthetic oral fluid, standards, controls, and patient specimens to appropriately labeled 13 x 100 mm test tubes.
2. Add 25 mcL Cannabinoid Panel *Working* Internal Standard to each test tube; vortex briefly to mix.
3. Add 2.0 mL DI water to each test tube; vortex briefly to mix.
4. Place a corresponding number of appropriately labeled 12 x 75 mm collection test tubes in the racks for each sample tube. Place a corresponding number of Oasis HLB 60mg extraction columns (Catalog number WAT094226) in the SPE Ware rack for each test tube to be extracted.
5. Condition columns with 2.0 mL Methanol; aspirate slowly through column.

6. Equilibrate columns with 2.0 mL Deionized Water; aspirate slowly through column.
7. Transfer prepared samples to columns using a Pasteur pipette; aspirate slowly through column.
8. Add 2.0 mL DI water to rinse the columns; aspirate slowly through column.
9. Add 2.0 mL 1.0 M Ammonium Carbonate, pH 10 to rinse the columns; aspirate slowly through column.
10. Add 2.0 mL hexane to rinse the columns; aspirate slowly through column.
11. Elute with 1.0 mL acetonitrile. Repeat this step.
12. Evaporate to dryness at 30±5°C using the TurboVap. Start with low nitrogen flow and increase as solvent evaporates. Do not leave dry tubes in TurboVap for an extended period of time.
13. Reconstitute by adding 250 mcL 40% LC-MS Mobile Phase A, 0.1% Formic acid in Water/ 60% LC-MS Mobile Phase B, 0.1% Formic acid in Methanol; vortex thoroughly to mix.
14. Transfer to appropriately labeled amber glass autosampler vials with glass inserts and cap with Teflon-lined snap-caps, or equivalent. Extracts are ready for LC-MS/MS analysis.

Analyte MRM Transitions

Analyte	Quant Ion	Ratio Ion
AB-FUBINACA-d4	373.07 > 256.97	395.10 > 350.10
5F-ADBICA	362.12 > 232.06	362.12 > 143.98
AB-FUBINACA	369.069 > 252.97	391.1 > 346.1
5F-ADB-PINACA	363.12 > 232.99	363.12 > 144.96
ADB-FUBINACA	383.085 > 253.015	383.08 > 109.01
AB-PINACA-D9	340.05 > 224.00	340.05 > 145.00
AB-PINACA	331.05 > 215.01	331.05 > 89.84
ADBICA	344.19 > 214.07	344.19 > 143.99
ADB-PINACA-D9	354.26 > 223.93	354.26 > 308.93
ADB-PINACA	345.25 > 214.93	345.25 > 299.93
AB-CHMINACA	357.1893 > 214.0423	357.1893 > 89.835
PB-22-D9	368.2 > 223.2	368.2 > 144.1
5F-PB-22	377.3 > 232.1	377.3 > 144.1
PB-22	359.2 > 214.2	359.2 > 144.1
BB-22	385.3 > 240.1	385.3 > 144
AM-2201-D5	365.2 > 155.1	365.2 > 127.0
AM-2201	360.2 > 155.1	360.2 > 127.0
XLR-11-D5	335.3 > 125.1	335.3 > 237.2
FUBIMINA	361.07 > 154.98	361.07 > 126.96

THJ-2201	360.94 > 233.01	360.94 144.97
XLR-11	330.3 > 125.1	330.3 > 232.2
5F-APICA	383.3 > 135.1	383.3 > 107.1
JWH-018-D9	351.3 > 155.1	351.3 > 127.1
JWH-018	342.3 > 155.1	342.3 > 127.1
5F-APINACA	384.3 > 135.1	384.3 > 79
APICA	365.3 > 135.1	365.30 > 214.10
JWH-081-D9	381.3 > 185.0	381.3 > 223.2
JWH-081	372.3 > 185.0	372.3 > 157.1
5F-AB-001	368.01 > 135.02	368.01 > 92.96
JWH-122-D9	365.3 > 169.0	365.3 > 223.2
THJ-018	343.01 > 215.04	343.01 > 144.96
JWH-122	356.3 > 169	356.3 > 141.1
UR-144-D5	317.3 > 125.1	317.3 > 219.2
UR-144	312.3 > 125.1	312.3 > 214.2
JWH-210-D9	379.4 > 183.1	379.4 > 223.2
JWH-210	370.4 > 183.1	370.4 > 214.2
APINACA-D9 (AKB-48-D9)	375.3 > 135.1	375.3 > 107.1
AB-001	350.3 > 135.1	350.3 > 93
APINACA	366.3 > 135.1	366.3 > 107.1

Gradient:

Time (min)	Flow Rate	%A	%B
Initial	0.4	55	45
4.00	0.4	15	85
5.50	0.4	5	95

Appendix B

LC-QTOF Broad Based Screening Method in Blood, Urine and Oral Fluid

Analytes included in panel: 1142 included in the overall scope of the instrumentation

INSTRUMENT PARAMETERS:

1. Instrumentation - Waters Xevo G2-S QTOF with an ACQUITY UPLC
2. Column – ACQUITY HSS C18 150x2.1mm 1.8 micron column
3. Mobile phase: LC-MS Mobile Phase
   a. A1: 5mM ammonium formate (pH 3.0)
   b. B2: 0.1% formic acid in acetonitrile
   c. Weak Wash: 5mM ammonium formate (pH 3.0)
   d. Strong Wash: 5:95 H_2O:ACN with 0.1% formic acid

PROCEDURE

Controls

An extracted control containing the following compounds was run with each sample set. The control was injected every 10 samples.

Compound	Concentration in Saliva(ng/mL)
	Low
2C-B	25
4-Fluoroamphetamine (4-FA)	75
alpha-PVP	2
Benzoylecgonine	1,000
Cocaethylene	10
Cocaine	5
Dimethylone	10
DOM	25
Ketamine	5
MDA	25
MDMA	25
Methamphetamine	50
Methylone	25
Norketamine	25
O-Desmethyltramadol	5
Tramadol	25

Appendix B

Internal Standard

Component Name	Chemical Formula	M+H Mass
D3-Morphine	C17H16D3NO3	289.1623
D3-Methylone	C11D3H10NO3	211.1153
D5-Alprazolam	C17H8D5N4Cl	314.1210
D5-MDMA	C11H10D5NO2	199.1484

Samples

Urine Samples Only:

1. Add 275 µL synthetic urine to 13x100 test tubes
2. Add 200 µL 1.0M acetate buffer, pH 5.5
3. Vortex
4. Spike the VSS samples:
    1. VSS A: 20 uL
    2. VSS B: 20 µL
    3. VSS C: 20 µL
    4. VSS D: 15 µL
5. Spike 10 µL hydrolysis control
6. Add 50 µL ISTD
7. Add 25 µL beta-glucuronidase (type 2, from Helix Pomatia)
8. Vortex
9. Cap and incubate at 55°C for approx. 1 hour
   (Meanwhile, proceed to steps 11-13)
10. Remove and allow to come to room temperature

Blood Samples Only:

11. Add 0.5mL sheep's blood to 13x100 test tubes
12. Spike the VSS samples:
    1. VSS A: 20 µL
    2. VSS B: 20 µL
    3. VSS C: 20 µL
    4. VSS D: 15 µL
13. Add 50 µL ISTD
    (Wait for urines to proceed)

All Samples:

14. Add 1.0mL of 0.1M Borax buffer, pH 10.4
15. Add 3.0mL 70/30 N-butyl chloride/ethyl acetate

16. Cap and rotate 5-10 min
17. Centrifuge 3750 RPM 10-15 minutes
18. Uncap and place rack in dry ice/acetone bath
19. Pour over into 13x100 test tubes
20. Turbovap 35°C ~10psi 15-20 minutes or until dry
21. Reconstitute with 200 µL 9:1 MPA:MPB
22. Vortex approximately 20 sec before vialing

Gradient

Time (min)	Flow (mL/min)	%A	%B
Initial	0.4	87	13
0.5	0.4	87	13
10	0.4	50	50
10.75	0.4	5	95
12.25	0.4	5	95
12.5	0.4	87	13
15	0.4	87	13

Appendix B

THC in Oral Fluid

Analytes included in panel and reporting limit:

Analyte	Reporting Limit
THC	2 ng/mL

INSTRUMENT PARAMETERS:

1. Instrumentation - Agilent 1100 Series Liquid Chromatograph coupled with an Agilent 6430 Tandem Mass Spectrometer
2. Column – Agilent Eclipse Plus C18 (3.5µm, 4.6 x 100 mm)
3. Mobile phase: LC-MS Mobile Phase
   a. A1: 0.1% formic acid in DI water
   b. B2: 0.1% formic acids in acetonitrile
   c. Needle Wash: Methanol

PROCEDURE

Calibrators and Controls

Preparation of Working Solution

Analyte	Stock Conc. (ng/µL)	Volume of Stock (µL)	Final Volume (µL)	Final Conc. (ng/mL)
THC	1,000	100	10,000	10

Oral Fluid THC Confirmation *Working* Calibrators and Controls

Transfer the following amounts as outlined by the table below to an appropriately labeled 13 x 100 mm test tube that contains 1 mL of oral fluid; vortex thoroughly to mix. Transfer that 1 mL of spiked oral fluid to 3 mL of quantisal collection buffer, sample 0.5 mL of the Quantisal/sample mixture and add that to a new labeled 13X100 test tube. Continue to "Samples" instructions.

Standard/QC	Volume Spiked (µL)	Sub Stock (ng/ µL)	Final OF Volume (µL)	Final Conc. (ng/mL)	ISTD Volume (µL)
Standard 1	20	50	1000	1000	100
Standard 2	60	10	1000	600	100
Standard 3	40	10	1000	400	100
Standard 4	10	10	1000	100	100
Standard 5	40	1	1000	40	100
Standard 6	20	1	1000	20	100
Standard 7	40	0.1	1000	4	100
Standard 8	20	0.1	1000	2	100
Negative	-	-	1000	-	100
Low QC	60	0.1	1000	6	100
High QC	80	10	1000	800	100

Appendix B

Samples

1. Add 1mL of pooled, blank oral fluid to calibrator and control test tubes using P1000µL pipette
2. Add appropriate volume of spiking mixes (calibrator and control, see above) using a syringe
3. Vortex all test tubes for approximately 15 seconds
4. Transfer (pour) oral fluid to corresponding tubes with 3 mL of Quantisal™ buffer
5. Vortex all tubes for approximately 30 seconds
6. Aliquot 0.5mL of calibrators, controls, and patient samples to appropriately labeled test tubes for extraction using P1000µL pipette set to 500µL
7. Add 100µL of internal standard mix (1ng/µL THC-D3) to all test tubes using repeat pipette
8. Vortex all test tubes for approximately 15 seconds
9. Add 1mL of 5% phosphoric acid in DI water to all test tubes
10. Vortex all test tubes for approximately 15 seconds
11. Add 3mL of extraction solvent (80:10:10 hexane, ethyl acetate, MTBE) to all test tubes
12. Cap all test tubes and rotate using rotator for 15 minutes
13. Centrifuge all test tubes at 4600 rpm for 15 minutes
14. Freeze/pour procedure using acetone and dry ice (or place in -80°C freezer for 15 minutes)
15. Transfer (pour) organic layer to appropriately labeled test tubes, avoiding the emulsion layer
16. Evaporate solvent in the TurboVap at 40°C for 30 minutes
17. Reconstitute sample in all test tubes using 100µL of 10:90 mobile phase A:B
18. Transfer samples to appropriate labeled autosampler vials using glass pipettes
19. Cap autosampler vials using snap caps
20. Transfer autosampler vials to instrument for analysis by LC/MS/MS

Analyte MRM Transitions

Analyte	Quant Ion	Ratio Ion
THC	315.1>193.1	315.1>259.2
THC-D3	318.1>196.1	318.1>262.1

Gradient

Time (min)	Flow Rate (mL/min)	% Mobile Phase A	% Mobile Phase B
0	1	10	90
5.5	1	10	90
6	1	90	10
6.5	1	90	10
7	1	10	90

Appendix B

Cannabinoids in Urine

Analytes included in panel and reporting limit:

ANALYTE	Reporting Limit
Delta-9-Carboxy THC	5.0 ng/mL

INSTRUMENT PARAMETERS:

1. **Instrumentation:** Agilent 6890 GC / 5975 MS
2. **Column:** DB-5MS capillary column, 20m x 0.18mm x 0.18 μm film thickness
3. **Injection Mode:** Splitless
4. **Injection Volume:** 1-3 μL
5. **Injection Temperature:** 275°C
6. **GC Oven Programming:**
   a. Initial Temperature = 150°C (1.0 min hold)
   b. Ramp = 30°C/min to 300°C (5.0 min hold)

PROCEDURE:

Technical Notes:

1. Working Solution I: Dilute the stock solution 1:100 with absolute ethanol and store frozen
2. Urine Control is Liquicheck C3 Urine Toxicology Confirm Control purchased form BioRad
3. Using 2 mL of certified negative urine, Prepare a five-point calibration curve according to the following chart:

Calibrator	Working Solution	Amount of Mixing Spiking Standard to spike (μL)	Final Concentration
Level 1	I	10	5 ng/mL
Level 2	I	30	15 ng/mL
Level 3	I	100	50 ng/mL
Level 4	I	200	100 ng/mL
Level 5	I	400	200 ng/mL
Negative	---	--	--

4. Add 2 mL of calibrators, controls and specimens to appropriately labeled 15 mL tubes.
5. Add 50 μL of Internal Standard Solution to all tubes
6. Add 400 μL KOH to all tubes, vortex well and incubate at 70 °C for 10 minutes.
7. Remove all samples and allow them to cool to room temperature

8. Add 4 mL of hexane: ethyl acetate (7:1) solution, cap and rotate all samples on a mixer for 10 minutes, then centrifuge at 3000 rpm for 5 minutes
9. Aspirate the organic phase to waste
10. Add 1 mL of concentrated HCl to all tubes and vortex well and then adjust the pH to 1-3 with KOH or HCl if necessary
11. Add 4 mL of hexane: ethyl acetate (7:1) solution, cap and rotate all samples on a mixer for 30 minutes, then centrifuge at 3000 rpm for 5 minutes
12. Transfer organic phase to 7 mL tubes and dry down using the N-EVAP at 55 °C
13. Add 100 µL of TMAH:DMSO (50µL:1mL) to all samples and vortex
14. Add 10 µL of iodomethane to all samples, vortex and allow tubes to stand for 3 minutes
15. Add 750 µL of 0.1 N HCl and 2 mL of isooctane to all tubes
16. Shake the tubes for 20 sec and then allow them to equilibrate for 5 minutes
17. Transfer the organic phase to 7 mL tubes and dry down using the N-EVAP at 55 °C
18. Reconstitute the samples in 50 µL of isooctane, vortex and transfer to autosampler vials

Analyte	Ions Monitored			Acceptance Range
	Target	Q1	Q2	
D3-THCC	316	360	--	±20%
THCC	313	357	372	±20%

Appendix B

Psilocin-TMS in Urine

Analytes included in panel reporting limit:

Analyte	Reporting Limit
Psilocin	0.005 mg/L

INSTRUMENT PARAMETERS:

7. **Instrumentation:** Agilent 6890 GC / 5975 MS
8. **Column:** DB-5MS capillary column, 20m x 0.18mm x 0.18 μm film thickness
9. **Injection Mode:** Split (10:1 to 50:1 ratio)

Analyte	Ions Monitored			Acceptance Range
	Quant Ion	Qualifier Ion 1	Qualifier Ion 2	
Psilocin	290	348	58	±20%
Psilocin-d10	358	292	-	±20%

PROCEDURE:

Calibration:

1. Prepare working solution I as follows: add 1.0 mL of psilocin calibrator stock standard (0.1 mg/mL) to 5 mL methanol and qs to 10 mL with methanol. Store frozen.
2. Prepare working solution II as follows: add 1.0 mL of psilocin Control Stock Standard (0.1 mg/mL) to 5 mL methanol and qs to 10 mL with methanol. Store frozen.
3. Using 3 mL of certified negative urine, prepare the calibrator and controls according to the following chart:

Sample	Working Solution	Standard Amount (μL)	Final Concentration
Calibrator	I	30	100 ng/mL
Negative Control	None	0	0 ng/mL
Low Control	II	15	50 ng/mL
High Control	II	90	300 ng/mL

4. Add 3 mL of calibrator, controls and specimens to appropriately labeled 16 x 100 mm tubes.
5. Add 50 μL of internal standard solution (0.01 mg/mL) to each tube.
6. Add 2 mL of 0.1 M phosphate buffer (pH 6.0) to each tube and vortex each tube.
7. To Urine samples: Add 25 μL of 100,000 U/mL *E. coli* β-glucuronidase and incubate tubes for 90 minutes in a 45°C water bath.
8. Centrifuge all tubes at 3000 rpm for 10 minutes.

9. Condition the 10 mL ZCDAU020 Clean Screen columns:
    a. 3 mL methanol
    b. 3 mL DI H2O
    c. 2 mL 0.1 M phosphate buffer (pH 6.0)
10. Load the samples onto the columns.
11. Wash the columns:
    a. 2 mL DI H2O
    b. 1 mL 0.1 M acetic acid
    c. 2 mL methanol
12. Dry the column at 25 psi for 10 minutes
13. Elute the samples with 2 mL of dichloromethane:isopropanol:ammonium hydroxide (78:20:2). This elution solvent is to be prepared fresh daily.
14. Evaporate the samples to dryness at 55°C under N2.
15. Add 50 μL of MSTFA to each tube.
16. Cap, vortex, and incubate all samples at 70°C for 15 minutes.
17. Transfer the samples to autosampler vials for analysis.

Appendix B

Benzodiazepine Panel in Urine

Analytes included in panel and reporting limits:

Analyte	Reporting Limit
Nordiazepam	0.025 mg/L
Desalkylflurazepam	0.025 mg/L
Oxazepam	0.025 mg/L
Diazepam	0.025 mg/L
Lorazepam	0.025 mg/L
Midazolam	0.025 mg/L
7-aminoclonazepam	0.025 mg/L
Temazepam	0.025 mg/L
1-hydroxymidazolam	0.025 mg/L
Clonazepam	0.025 mg/L
Alprazolam	0.025 mg/L
1-hydroxyalprazolam	0.025 mg/L
1-hydroxytriazolam	0.025 mg/L

INSTRUMENT PARAMETERS:

1. **Instrumentation:** Agilent 6890 GC / 5975 MS
2. **Column:** RTX-200 capillary column, 30m x 0.25mm x 0.25 μm film thickness
3. **Injection Mode:** Split (2:1 ratio)

Analyte	Ions Monitored			Acceptance Range
	Quant Ion	Qualifier Ion 1	Qualifier Ion 2	
Nordiazepam	327	329	328	±20%
Desalkylflurazepam	345	346	347	±20%
Oxazepam	457	513	459	±20%
Diazepam	256	238	284	±20%
Lorazepam	491	513	493	±20%
Midazolam	310	312	325	±20%
7-aminoclonazepam	342	343	344	±20%
Temazepam	357	283	255	±20%
1-hydroxymidazolam	398	400	399	±20%
Clonazepam	372	374	373	±20%
Alprazolam	279	308	204	±20%
1-hydroxyalprazolam	381	383	382	±20%

1-hydroxytriazolam	415	417	416	±20%
Oxazepam-d5	462	519	-	±20%
Diazepam-d5	261	288	-	±20%
7-aminoclonazepam-d4	346	332	-	±20%
Clonazepam-d4	376	378	-	±20%
1-hydroxyalprazolam-d5	386	388	-	±20%

PROCEDURE:

Calibrators and Controls

1. Prepare working solution I as follows: add 100 µL of the control stock standard (containing diazepam and alprazolam at 1 mg/mL) to 10 mL volumetric flask and qs to volume with acetonitrile. Store frozen.
2. Prepare working solution II as follows: add 100 µL of each stock standard (1mg/mL) to a 10 mL volumetric flask and qs to volume with acetonitrile. Store frozen.
3. Prepare workings solutions III as follows: add 1 mL of the working solution II to a 10 mL volumetric flask and qs to volume with acetonitrile. Store frozen.
4. Urine control solution (0.01 mg/mL): Add 1 mL of oxazepam-glucuronide and lorazepam-glucuronide (0.1mg/mL) to a 10 mL volumetric flask and fill to volume with acetonitrile. Store frozen.
5. Using 1 mL of certified negative urine, prepare the calibrator and controls according to the following chart:

Sample	Working Solution	Standard Amount (µL)	Final Concentration
Calibrator 1	III	25	25 ng/mL
Calibrator 2	III	50	50 ng/mL
Calibrator 3	III	100	100 ng/mL
Calibrator 4	II	25	250 ng/mL
Calibrator 5	II	50	500 ng/mL
Calibrator 6	II	100	1000 ng/mL
Negative Control	None	None	0 ng/mL
Low Control	I	20	200 ng/mL
High Control	I	75	750 ng/mL
Hydrolysis Control	Urine Control	80	800 ng/mL

6. Add 1 mL of calibrator, controls and specimens to appropriately labeled 16 x 100 mm tubes.

7. Add 20 μL of internal standard solution (0.01 mg/mL) to each tube.
8. Add 2 mL of 0.1 M sodium acetate buffer (pH 4.5) to each tube and vortex each tube.
9. To Urine samples: Add 50 μL of 100,000 U/mL *E. coli* β-glucuronidase and incubate tubes for 30 minutes in a 70°C water bath.
10. Centrifuge all tubes at 3000 rpm for 10 minutes.
11. Condition the Phenomenex Strata-XC columns:
    a. 1 mL elution solvent (2% ammonium hydroxide in ethyl acetate)
    b. 3 mL methanol
    c. 3 mL DI H2O
    d. 1 mL 0.1 M acetate buffer (pH 4.5)
12. Load the samples onto the columns.
13. Wash the columns:
    a. 2 mL DI H2O
    b. 2 mL 20% acetonitrile in 0.1M acetate buffer
14. Dry the column at 25 psi for 20 minutes
15. Wash the columns:
    a. 2 mL hexane
16. Elute the samples with 3 mL of elution solvent. This elution solvent is to be prepared fresh daily.
17. Evaporate the samples to dryness at 40°C under N2.
18. Add 40 μL of 4:1 Acetonitrile:MTBSTFA w/1% TMBDMS to each tube.
19. Vortex and transfer the samples to autosampler vials for analysis.

Appendix B

Opiate Panel in Urine

Analytes included in panel:
Codeine, Morphine, Hydromorphone, Hydrocodone, Oxycodone, Oxymorphone

Reporting Limits:

Analyte	Reporting Limit
Codeine	0.025 mg/L
Morphine	0.025 mg/L
Hydromorphone	0.025 mg/L
Hydrocodone	0.025 mg/L
Oxycodone	0.025 mg/L
Oxymorphone	0.025 mg/L

INSTRUMENT PARAMETERS:

1. **Instrumentation:** Agilent 6890 GC / 5975 MS
2. **Column:** DB-1MS capillary column, 30m x 0.25mm x 0.25 μm film thickness
3. **Injection Mode:** Split (5:1 to 20:1 ratio)

Analyte	Ions Monitored			Acceptance Range
	Quant Ion	Qualifier Ion 1	Qualifier Ion 2	
Codeine	371	343	372	±20%
Morphine	429	430	401	±20%
Hydrocodone	386	297	387	±20%
Hydromorphone	444	355	429	±20%
Oxycodone	474	475	459	±20%
Oxymorphone	532	517	533	±20%
Codeine d3	371	346	-	±20%
Morphine d3	432	417	-	±20%
Hydrocodone d3	389	374	-	±20%
Hydromorphone d3	447	358	-	±20%
Oxycodone d3	477	462	-	±20%
Oxymorphone d3	535	520	-	±20%

PROCEDURE:
Calibrators and Controls:

1. Prepare working solution I as follows: add 1 mL of the calibrator stock standard (containing analytes at 1 mg/mL) to 10 mL volumetric flask and qs to volume with methanol. Store frozen.
2. Prepare working solution II as follows: add 1 mL of working solution I (0.1mg/mL) to a 10 mL volumetric flask and qs to volume with methanol. Store frozen.

3. Prepare workings solutions III as follows: add 1 mL of the working solution II to a 10 mL volumetric flask and qs to volume with acetonitrile. Store frozen.
4. Urine control solution: Liquichek C3 Urine Toxicology Confirm Controls is purchased from BioRad.
5. Using 2 mL of certified negative urine, prepare the calibrator and controls according to the following chart:

Sample	Working Solution	Standard Amount (μL)	Final Concentration
Calibrator 1	II	10	50 ng/mL
Calibrator 2	II	20	100 ng/mL
Calibrator 3	II	40	200 ng/mL
Calibrator 4	II	100	500 ng/mL
Calibrator 5	I	30	1500 ng/mL
Calibrator 6	I	60	3000 ng/mL

6. Add 2 mL of calibrator, controls and specimens to appropriately labeled 16 x 100 mm tubes.
7. Add 100 μL of internal standard solution (0.01 mg/mL) to each tube.
8. Add 1 mL of concentrated HCl to all tubes. Autoclave these samples using a 15psi x 45 minute Liquid Cycle.
9. Remove these samples from the autoclave and allow them to cool to room temperature.
10. Add between 0.85-0.90 mL of concentrated KOH to these tubes
11. Add 2 mL of 0.3M phosphate buffer (pH6.0) to these tubes. Adjust pH to 6.0 using 2M HCl or KOH.
12. Add 250 μL of 10% hydroxylamine to all tubes
13. Incubate tubes for 15 minutes at 70°C.
14. Centrifuge all tubes at 3000 rpm for 10 minutes.
15. Condition the UCT ZCDAU020 Clean Screen columns:
    a. 3 mL methanol
    b. 2 mL DI H2O
    c. 2 mL 0.3 M phosphate buffer (pH 6.0)
16. Load the samples onto the columns.
17. Wash the columns:
    a. 2 mL DI H2O
    b. 2 mL 0.1M acetic acid
    c. Dry the columns for 2 minutes
    d. 3 mL methanol
18. Dry the column for 10 minutes
19. Elute the samples with 3 mL 2% ammonium hydroxide in ethyl acetate:methanol (2:1). This elution solvent is to be prepared fresh daily.
20. Evaporate the samples to dryness at 55°C under N2.
21. Add 50 μL of acetonitrile to each tube.
22. Add 25 μL of BSTFA with 1% TMCS to each tube.
23. Incubate all tubes at 70°C for 35 minutes.
24. Centrifuge all tubes at 3000 rpm for 5 minutes.
25. Vortex and transfer the samples to autosampler vials for analysis.

Appendix B

Cocaine and Metabolite Panel in Urine

Analytes included in panel reporting limits:

Analyte	Reporting Limit
Benzoylecgonine	0.010 mg/L
Cocaine	0.010 mg/L
Cocaethylene	0.010 mg/L

INSTRUMENT PARAMETERS:

1. **Instrumentation:** Agilent 6890 GC / 5975 MS
2. **Column:** DB-5MS capillary column, 20m x 0.18mm x 0.18 μm film thickness
3. **Injection Mode:** Pulsed Splitless

Analyte	Ions Monitored			Acceptance Range
	Quant Ion	Qualifier Ion 1	Qualifier Ion 2	
Benzoylecgonine	300	316	421	±20%
Cocaine	182	303	198	±20%
Cocaethylene	196	317	272	±20%
Benzoylecgonine d3	303	424	-	±20%
Cocaine d3	185	306	-	±20%
Cocaethylene d8	204	325	-	±20%

PROCEDURE:

Calibrators and Controls:

1. Prepare working solution I as follows: add 1 mL of the calibrator stock standard (containing analytes at 1 mg/mL) to 10 mL volumetric flask and qs to volume with acetonitrile. Store frozen.
2. Prepare working solution II as follows: add 1 mL of working solution I (0.1mg/mL) to a 10 mL volumetric flask and qs to volume with acetonitrile. Store frozen.
3. Urine control solution: Liquichek C4 Urine Toxicology Confirm Controls is purchased from BioRad.
4. Using 1 mL of certified negative urine, prepare the calibrator and controls according to the following chart:

Sample	Working Solution	Standard Amount (μL)	Final Concentration
Calibrator 1	II	10	10 ng/mL

Calibrator 2	II	25	25 ng/mL
Calibrator 3	II	50	500 ng/mL
Calibrator 4	II	100	100 ng/mL
Calibrator 5	I	50	500 ng/mL
Calibrator 6	I	100	1000 ng/mL

5. Add 1 mL of calibrator, controls and specimens to appropriately labeled 16 x 100 mm tubes.
6. Add 25 μL of internal standard solution (0.01 mg/mL) to each tube.
7. Add 2 mL of 0.1 M phosphate buffer (pH6.0)
8. Condition the UCT ZCDAU020 Clean Screen columns:
    a. 3 mL methanol
    b. 3 mL DI H2O
    c. 3 mL 0.1 M phosphate buffer (pH 6.0)
9. Load the samples onto the columns.
10. Wash the columns:
    a. 3 mL DI H2O
    b. 3 mL 0.1 M HCl
    c. 3 mL methanol
11. Dry the column for 10 minutes
12. Elute the samples with 3 mL dichloromethane:Isopropanol:ammonium hydroxide (78:20:0). This elution solvent is to be prepared fresh daily.
13. Evaporate the samples to dryness at 55°C under N2.
14. Add 50 μL of PFPOH and 50 μL of PFPA to each tube.
15. Incubate all tubes at 55°C for 30 minutes.
16. Reconstitute samples in 50 μL of ethyl acetate.
17. Vortex and transfer the samples to autosampler vials for analysis.

Appendix B

Amphetamines Panel in Urine

Analytes included in panel and reporting limits:

Analyte	Reporting Limit
Amphetamine	0.05 mg/L
Methamphetamine	0.05 mg/L
MDA	0.05 mg/L
MDMA	0.05 mg/L
Phenylpropanolamine	0.05 mg/L
Pseudoephedrine	0.05 mg/L
Ephedrine	0.05 mg/L

INSTRUMENT PARAMETERS:

1. **Instrumentation:** Agilent 6890 GC / 5975 MS
2. **Column:** DB-5MS capillary column, 30m x 0.25mm x 0.25 μm film thickness
3. **Injection Mode:** Split (10:1 to 25:1 ratio)

Analyte	Ions Monitored			Acceptance Range
	Quant Ion	Qualifier Ion 1	Qualifier Ion 2	
Amphetamine	156	118	91	±20%
Methamphetamine	170	118	91	±20%
Pseudoephedrine	260	126	170	±20%
Phenylpropanolamine	156	158	246	±20%
Ephedrine	260	126	170	±20%
MDA	135	162	291	±20%
MDMA	170	305	162	±20%
Amphetamine d11	160	128	-	±20%
Methamphetamine d14	177	179	-	±20%
Pseudoephedrine d3	263	173	-	±20%
Phenylpropanolamine d3	249	159	-	±20%
Ephedrine d3	263	173	-	±20%
MDA d5	167	296	-	±20%
MDMA d5	174	310	-	±20%

PROCEDURE:

Calibrators and Controls:

1. Prepare working solution I as follows: add 1 mL of the calibrator stock standard (containing analytes at 1 mg/mL) to 10 mL volumetric flask and qs to volume with methanol. Store frozen.
2. Prepare working solution II as follows: add 1 mL of working solution I (0.1mg/mL) to a 10 mL volumetric flask and qs to volume with methanol. Store frozen.
3. Urine control solution: Liquichek C3 Urine Toxicology Confirm Controls is purchased from BioRad.
4. Using 1 mL of certified negative urine, prepare the calibrator and controls according to the following chart:

Sample	Working Solution	Standard Amount (μL)	Final Concentration
Calibrator 1	II	10	10 ng/mL
Calibrator 2	II	25	25 ng/mL
Calibrator 3	II	50	500 ng/mL
Calibrator 4	II	100	100 ng/mL
Calibrator 5	I	50	500 ng/mL
Calibrator 6	I	100	1000 ng/mL

5. Add 2 mL of calibrator, controls and specimens to appropriately labeled 16 x 100 mm tubes.
6. Add 100 μL of internal standard solution to each tube.
7. Add 4 drops of concentrated KOH to each tube.
8. Add 5 mL of chlorobutane to each tube
9. Rotate all tubes on a mixer at low speed for 15 minutes.
10. Centrifuge all tubes at 3000 rpm for 10 minutes
11. Transfer the upper organic layer to appropriately-labeled 13x100 mm tubes. Add 50 μL of 10% HCl in methanol to each tube.
12. Evaporate the samples to dryness at 55°C under N2.
13. Reconstitute all extracts with 100 μL of ethyl acetate and 25 μL of chlorodifluoroacetic anhydride and then vortex, cap and incubate at 70°C for 15 minutes.
14. Remove all samples and evaporate to dryness.
15. Reconstitute samples in 100 μL of ethyl acetate.
16. Vortex and transfer the samples to autosampler vials for analysis.

Appendix B

1,3-Dimethylamylamine (DMAA) Analysis in Urine

Analyte included in panel and reporting limit:

Analyte	Reporting Limit
DMAA	10 ng/mL

INSTRUMENT PARAMETERS:

1. **Instrumentation:** Agilent 1100 series LC / 3200 Sciex QTrap MS/MS System
2. **Column:** XDB C18 column, 75mm x 4.6mm x 3μm with Halo C18 guard column
3. **Injection Volume:** 1-10 μL
4. **Column Temperature:** 35°C

PROCEDURE:

Calibrators and Controls:

1. Prepare working solution I as follows: a serial dilution of 1:1000 is performed on the reference standard (10 mg/mL) for a final concentration of 10 ng/μL in ethanol. Store frozen.
2. Prepare working solution II as follows: a serial dilution of 1:10 is performed on the working solution I for a final concentration of 1 ng/μL in ethanol. Store frozen.
3. Using 0.5 mL of certified negative urine, prepare the calibrator and controls according to the following chart:

Sample	Working Solution	Standard Amount (μL)	Final Concentration
Calibrator 1	II	12.5	25 ng/mL
Calibrator 2	II	25	50 ng/mL
Calibrator 3	II	50	100 ng/mL
Calibrator 4	I	12.5	250 ng/mL
Calibrator 5	I	25	500 ng/mL
Negative Control	None	0	0 ng/mL
Positive Control	I	17.5	350 ng/mL

4. Add 0.5 mL calibrator/control/sample to labeled 16 x 100mm tubes.
5. Add 25 μL of Internal Standard Working Solution to all tubes.
6. Add 4 drops of concentrated KOH
7. Add 4 mL of chlorobutane to all tubes, cap and vortex.
8. Mix tubes for 20 min.
9. Centrifuge all tubes for 10 min @ 3000 rpm.
10. Transfer the organic layer (upper) into 16 x 100mm conical centrifuge tube.

11. Add 25 µL of methanolic HCL (10%)
12. Evaporate to dryness @ 55°C under N_2 (~5-8 psi).
13. Reconstitute the extract in 150 µL of mobile phase and vortex.
14. Transfer samples to polypropylene autosampler vials.

Analyte MRM Transitions

Analyte	Quant Ion	Ratio Ion
DMAA	116.1>57.0	116.1>99.1
Amphetamine-d8	144.2>97.1	144.2>127.2

Gradient

Time (min)	Flow Rate (mL/min)	% Mobile Phase A	% Mobile Phase B
0	0.8	50	50
3	0.8	50	50
5	0.8	20	80

Appendix B

Rapid Fire Synthetic Cannabinoid Screen in Urine

Analytes included in panel with reporting limits:

Analyte	Reporting Limit
JWH 073 N-4-COOH/JWH 018 N-5-OH	5.0 ng/mL
JWH 073 N-4-OH	5.0 ng/mL
JWH 018 N-5-COOH	5.0 ng/mL
AM 2201 N-4-OH	5.0 ng/mL
JWH 122 N-5-OH	5.0 ng/mL
JWH 081 N-5-OH	5.0 ng/mL
JWH 250 N-5-COOH	5.0 ng/mL
JWH 250 N-5-OH	5.0 ng/mL
RCS 4 N-5-COOH	5.0 ng/mL
XLR 11 N-4-OH	5.0 ng/mL
UR 144 N-5-COOH	5.0 ng/mL
MAM 2201 N-5-COOH (JWH 122 N-5-COOH)	5.0 ng/mL
PB 22 3-Carboxyindole	5.0 ng/mL
5F PB 22 3-Carboxyindole	5.0 ng/mL
ADBICA N-5-COOH	5.0 ng/mL
ADB PINACA N-5-COOH	5.0 ng/mL
AKB48 N-5-COOH	5.0 ng/mL
AD Fubinaca COOH	5.0 ng/mL

INSTRUMENT PARAMETERS:

1. **Instrumentation:** Agilent RapidFire 365, Agilent 6460 MS/MS
2. **Column:** A2 (C4) Cartridge
3. **RapidFire States:**
    a. Aspirate – 600 ms
    b. Load – 1500 ms
    c. Extra Wash – 5000 ms
    d. Elute – 5000 ms
    e. Re-equilibrate – 500 ms
4. **Buffer A:** 10mM Ammonium Acetate with 0.1% formic acid at 1.5 mL/min
5. **Buffer B:** 50:50 Water:MeOH with 0.1% formic acid at 2.0 mL/min
6. **Buffer C:** 85:15 Ethyl acetate:isopropanol at 1.25 mL/min
7. **Source Parameters:**
    a. **Gas Temperature –** 300°C
    b. **Gas flow rate** – 13 L/min
    c. **Nebulizer** – 50 psi
    d. **Sheath gas temperature** – 300°C
    e. **Sheath gas flow** – 11 L/min
    f. **Nozzle voltage** – 1000 V
    g. **Capillary voltage** – 3000 V

h. **CAV** – 3 V
i. **Dwell** – 10 ms

PROCEDURE:

Calibrators:

1. Prepare working solution I as follows: add 15 ng/mL of 5F PB22 Carboxyindole and 3.75 ng/mL of all other listed synthetic cannabinoid metabolites in negative urine
2. Prepare working solution II as follows: add 25 ng/mL of 5F PB 22 Carboxyindole and 6.25 ng/mL of all other listed synthetic cannabinoid metabolites in negative urine
3. Place 50 µL of calibrator, controls, and specimens in individual wells of 96-well plate according to batch setup.
4. Add 25 µL of internal standard solution to each well.
5. Add 25 µL of 0.3N NaOH to all wells and mix. Incubate at room temperature for 15 minutes.
6. Add 150 µL of diluent to all wells and mix.
7. Seal plate with plate loc sealer.
8. Centrifuge @ 3000 rpm for 5 minutes.

Analyte	MRM Transition	CE	Fragmentor
JWH 073 N-4-COOH/JWH 018 N-5-OH	358.2/127.0	50	131
JWH 073 N-4-OH	344.2/155.0	18	136
JWH 018 N-5-COOH	372.2/155.0	18	131
AM 2201 N-4-OH	376.2/155.0	18	131
JWH 122 N-5-OH	372.2/169.0	18	136
JWH 081 N-5-OH	388.2/185.0	18	141
JWH 250 N-5-COOH	366.2/121.1	18	124
JWH 250 N-5-OH	352.2/121.1	18	119
RCS 4 N-5-COOH	352.2/135.0	18	131
XLR 11 N-4-OH	346.2/248.1	18	116
UR 144 N-5-COOH	342.2/125.1	18	131
MAM 2201 N-5-COOH (JWH 122 N-5-COOH)	386.2/169.0	22	146
PB 22 3-Carboxyindole	232.1/132.1	14	104
5F PB 22 3-Carboxyindole	250.1/118.1	22	114
AKB48 N-5-COOH	396.2/135.1	18	104
ADB PINACA N-5-COOH	375.2/330.1	10	99
ADBICA N-5-COOH	374.2/144.0	38	94
AD Fubinaca COOH	399.1/253.0	22	94
JWH 073 N-4-COOH-D5 (ISTD)	363.2/155.0	18	126

Appendix B

Rapid Fire Bath Salts Screen in Urine

Analytes included in panel and reporting limits:

Analyte	Reporting Limit
25-I-NBOMe	10.0 ng/mL
MDPV	10.0 ng/mL
Pentylone	10.0 ng/mL
alpha-PVP	10.0 ng/mL
Butylone	10.0 ng/mL
Ethylone	10.0 ng/mL
alpha-PBP	10.0 ng/mL
Methylone	10.0 ng/mL
alpha-PPP	10.0 ng/mL
4-Methyl ethcathinone	10.0 ng/mL
Mephedrone	10.0 ng/mL
N-ethyl cathinone	10.0 ng/mL
Buphedrone	10.0 ng/mL
5-APB	10.0 ng/mL

INSTRUMENT PARAMETERS:

1. **Instrumentation:** Agilent RapidFire 365, Agilent 6460 MS/MS
2. **Column:** A2 (C4) Cartridge
3. **RapidFire States:**
    a. Aspirate – 600 ms
    b. Load – 3000 ms
    c. Extra Wash – 0 ms
    d. Elute – 5000 ms
    e. Re-equilibrate – 500 ms
4. **Buffer A:** 10mM Ammonium Acetate with 0.1% formic acid at 1.5 mL/min
5. **Buffer B:** N/A
6. **Buffer C:** 50:25:25 water:acetone:acetonitrile with 0.1% formic acid at 1.25 mL/min
7. **Source Parameters:**
    a. **Gas Temperature –** 300°C
    b. **Gas flow rate** – 11 L/min
    c. **Nebulizer** – 50 psi
    d. **Sheath gas temperature** – 300°C
    e. **Sheath gas flow** – 11 L/min
    f. **Nozzle voltage** – 500 V
    g. **Capillary voltage** – 3000 V
    h. **CAV** – 3 V
    i. **Dwell** – 15 ms

Appendix B

PROCEDURE:

Calibrators:

1. Prepare working solution I as follows: add 7.5 ng/mL of all listed bath salts in negative urine
2. Prepare working solution II as follows: add 12.5 ng/mL of all listed bath salts in negative urine
3. Place 50 µL of calibrator, controls, and specimens in individual wells of 96-well plate according to batch setup.
4. Add 25 µL of internal standard solution to each well.
5. Add 25 µL of 0.3N NaOH to all wells and mix. Incubate at room temperature for 15 minutes.
6. Add 150 µL of diluent to all wells and mix.
7. Seal plate with plate loc sealer.
8. Centrifuge @ 3000 rpm for 5 minutes.

Analyte	MRM Transition	CE	Fragmentor
25-I-NBOMe	428.07/121	14	116
MDPV	276.16/126.1	22	116
Pentylone	236.13/188.1	14	91
alpha-PVP	232.17/126.1	22	116
Butylone	222.12/174.1	14	91
Ethylone	222.12/174.1	14	86
alpha-PBP	218.16/112.1	26	116
Methylone	208.1/160.1	14	96
alpha-PPP	204.14/105.1	22	116
4-Methyl ethcathinone	192.14/174.1	10	86
Mephedrone	178.13/160.1	10	86
N-ethyl cathinone	178.13/160.1	10	86
Buphedrone	178.16/160.1	10	86
5-APB	176.11/131	14	56
MDPV-d8 (ISTD)	284.21/175.1	18	116

Appendix B

Basic Drug Quantitation in Urine

Analytes included in panel and reporting limits:

Analyte	Reporting Limit
Buproprion	0.05 mg/L
Methylphenidate	0.05 mg/L
Fluoxetine	0.05 mg/L
Dextromethorphan	0.05 mg/L
Cyclobenzaprine	0.05 mg/L
Buprenorphine	10 ng/mL
Norbuprenorphine	10 ng/mL
Azacyclonal	25 ng/mL
Norketamine	0.05 mg/L
Dehydronorketamine	0.05 mg/L
Sildenafil	10 ng/mL
Quetiapine	0.05 mg/L
Norquetiapine	0.05 mg/L
Methylone	1 ng/mL
Ethylone	1 ng/mL
Butylone	1 ng/mL
4-Fluoroamphetamine	1 ng/mL
Alpha-PVP	1 ng/mL
Dimethylone	1 ng/mL

Method Information:

Analyte	Extraction Type	Instrument Type
Buproprion	SPE	GC/MS
Methylphenidate	SPE	GC/MS
Fluoxetine	SPE	GC/MS
Dextromethorphan	SPE	GC/MS
Cyclobenzaprine	SPE	GC/MS
Buprenorphine	SPE	GC/MS
Norbuprenorphine	SPE	GC/MS
Azacyclonal	SPE	GC/MS
Ketamine	SPE	GC/MS
Sildenafil	SPE	GC/MS
Quetiapine	SPE	GC/MS
Norquetiapine	SPE	GC/MS
Methylone	LLE	LC/MS/MS
Ethylone	LLE	LC/MS/MS
Butylone	LLE	LC/MS/MS
4-Fluoroamphetamine	LLE	LC/MS/MS
Alpha-PVP	LLE	LC/MS/MS
Dimethylone	LLE	LC/MS/MS

Appendix B

INSTRUMENT PARAMETERS:

1. **Instrumentation:** Agilent 1100 series LC / 3200 Sciex QTrap MS/MS System
2. **Column:** C18 column, 50-100mm x 4.6mm x 3μm with Halo C18 guard column
3. **Mobile Phase A:** 0.1% Formic acid in water
4. **Mobile Phase B:** 0.1% Formic acid in acetonitrile
5. **Injection Volume:** 1-10 μL
6. **Column Temperature:** 35°C

1. **Instrumentation:** Agilent 6890 GC / 5975 MS
2. **Column:** DB-5MS capillary column, 30m x 0.25mm x 0.25 μm film thickness
3. **Injection Mode:** Split (5:1 to 50:1 ratio)
4. **Injection Volume:** 1-3 μL
5. **Injection Temperature:** 260°C
6. **GC Oven Programming:**
    a. Initial Temperature = 110°C (1.0 min hold)
    b. Ramp #1 = 20°C/min to 200°C (1.0 min hold)
    c. Ramp #2 = 10°C/min to 300°C (1.0 min hold)

PROCEDURE:

Calibrators:

1. Calibration curves reflect the relevant and anticipated concentration range of the analyte(s) being analyzed. The calibration range should be pertinent to the method of analysis. Typical concentration ranges are 0.05 – 2.0 mg/L and 0.010 – 0.500 mg/L for GC/MS and LC/MS/MS respectively. A minimum of 5 calibrators must be prepared when extracting a multi-point calibration curve.

Samples LLE:

1. Add 2 mL calibrator/control/sample to labeled 16 x 100mm tubes.
2. Add 2 mL of sodium borate to all samples
3. Add an appropriate amount of internal standard to all samples
4. Add 500 μL of concentrated NH_4OH to all samples and ensure that the pH is >9.0
5. Add 7 mL of chlorobutane to all samples
6. Cap, vortex and rotate all samples on mixer for 20 minutes
7. Centrifuge @ 3000 rpm for 5 minutes
8. Transfer the organic layer to clean, labeled 16 x 125mm tubes
9. Add 3.0 mL 0.2 N H_2SO_4
10. Rotate all samples on mixer for 10 minutes
11. Centrifuge @ 3000 rpm for 5 minutes
12. Aspirate the organic layer to waste
13. Add 4 drops concentrated KOH to the aqueous layer of all samples and ensure that the pH is >10
14. Add 5 mL of chlorobutane to all samples

15. Rotate all samples on mixer for 10 minutes
16. Centrifuge @ 3000 rpm for 5 minutes
17. Transfer organic layer to conical tubes
18. Add 25 µL of 10% methanolic HCl to each tube
19. Evaporate to dryness under N_2 @ 55 °C
20. Reconstitute samples with 50 µL methanol (or 100 µL mobile phase for LC)
21. Transfer to autosampler vials for analysis

Samples SPE:

1. Add 2 mL calibrator/control/sample to labeled 16 x 100mm tubes.
2. Add an appropriate amount of internal standard to all samples
3. Add 2 mL of phosphate buffer (pH 6.0) to all samples
4. Cap and vortex all samples
5. Centrifuge @ 3000 rpm for 5 minutes
6. Condition 10 mL UCT clean screen columns
    a. 3 mL methanol
    b. 2 mL DI H_2O
    c. 2 mL 0.1 M phosphate buffer (pH 6.0)
7. Apply the samples to columns
8. Wash the columns
    a. 2 mL DI H_2O
    b. 2 mL 0.1 M acetic acid
    c. 3 mL methanol
9. Dry columns for 10 minutes
10. Elute the samples using 3 mL of dichloromethane:isopropanol:NH_4OH (78:20:2)
11. Add 100 µL of 1% HCl in methanol to each sample
12. Evaporate the samples to dryness at 40 °C
13. Reconstitute all samples in 50 µL ethyl acetate (or 100 µL mobile phase for LC/MS)
14. Centrifuge @ 3000 rpm for 5 minutes if precipitate forms in the samples
15. Transfer the samples to autosampler vials

Analyte MRM Transitions

Analyte	Quant Ion	Ratio Ion
Methylone	208.1>160.2	208.1>190.2
Ethylone	222.3>174.3	222.3>204.2
Butylone	222.2>174.0	222.2>131.1
4-Fluoroamphetamine	154.1>137.2	154.1>109.1
Alpha-PVP	232.2>126.2	232.2>104.9
Dimethylone	222.2>146.9	222.2>91.0
Methylone d3	211.2>163.3	211.2>135.2
Ethylone d5	227.2>179.1	227.2>209.1
MDPV d8	284.2>134.1	284.2>175.2

Appendix B

GC Ion Parameters

Analyte	Ions Monitored			Acceptance Range
	Quant Ion	Qualifier Ion 1	Qualifier Ion 2	
Buproprion	100	111	224	±20%
Methylphenidate	91	115	172	±20%
Fluoxetine	309	251	183	±20%
Dextromethorphan	271	214	171	±20%
Cyclobenzaprine	215	202	189	±20%
Fluoxetine d6	315	257		±20%
Buprenorphine	450	482	492	±20%
Norbuprenorphine	468	524	510	±20%
Buprenorphine d4	454	486		±20%
Azacyclonal	361	205	232	±20%
Ephedrine d3	263	173		±20%
Norketamine	284	275	256	±20%
Dehydronorketamine	317	282	214	±20%
Proadifen	165	99		±20%
Sildenafil	238	283	311	±20%
Vardenafil	489	151		±20%
Quetiapine d8	330	316		±20%
Quetiapine	322	321	308	±20%
Norquetiapine	227	210	239	±20%

Gradient

Time (min)	Flow Rate (mL/min)	% Mobile Phase A	% Mobile Phase B
0	0.8	90	10
0.5	0.8	90	10
10	0.8	10	90

Table of Contents: Appendix C-Validation Data

Appendix C

Validation Data-Methylone in Blood

Method Limitations	
Limit of Detection:	5 ng/mL
Limit of Quantitation:	5 ng/mL
Carryover <:	5000 ng/mL

Calibration Curve Information			
	Acceptability Criteria	Method Results	
Slope:	1 (0.95-1.05)	1.012	Acceptable
r2:	1 (0.98-1.00)	0.9991	Acceptable
Intercept:	0 (-0.5-0.5)	-0.005	Acceptable
Linear Dynamic Range:	5-500 ng/mL		
Calibration Range:	5-500 ng/mL		

Percent Recovery			
	Acceptability Criteria	Method Results	
Compound:	>70%	92.62%	Acceptable

Stability of Compound			
Processed Sample Stability			
	Time	Peak Area	Stability
Low Control	24 Hour	2887.66	Unstable
	48 Hour	2829.33	Unstable
	72 Hour	2888	Unstable
High Control	24 Hour	52323.33	Unstable
	48 Hour	56227.66	Unstable
	72 Hour	56862	Unstable

Ionization Suppression/Enhancement Percentage				
		Acceptability Criteria	Method Results	
Low Control:	Suppression or Enhancement	±25%	10.14%	Acceptable
	%CV	< 15%	8.46%	Acceptable
High Control:	Suppression or	±25%	-5.55%	Acceptable

	Enhancement			
	%CV	< 15%	5.03%	Acceptable

Matrix Match Acceptance					
Matrix:			Acceptability Criteria	Method Results	
Urine	Low Control (15 ng/mL)	Mean	±20% Target	15.0 (0%)	Acceptable
		%CV	<20%	4.73%	Acceptable
	High Control (350 ng/mL)	Mean	±20% Target	350.3 (0.08%)	Acceptable
		%CV	<20%	2.10%	Acceptable

Interferences			
	Interference?		Interference?
Matrix	NO	MDA	NO
Internal Standard with Drug	NO	MDMA	NO
Drug with Internal Standard	NO	MDPV	NO
2-C-T-7	NO	Methadone	NO
3,4 Dimethylmethcathinone	NO	Methamphetamine	NO
Acetaminophen	NO	Morphine	NO
Amphetamine	NO	Oxycodone	NO
Caffeine	NO	Tramadol	NO
Clonazepam	NO	O-Desmethyltramadol	NO
Cocaine	NO	Diazepam	NO
Codeine	NO	MDPP	NO
Cotinine	NO		

Appendix C

Method Bias, Precision and Accuracy				
Bias				
	Acceptability Criteria	Method Results		
LLOQ:	Mean Bias % of 5 Days (<20%)	11.73%		Acceptable
Low Control:		2.93%		Acceptable
High Control:		3.67%		Acceptable
Within Run Precision:				
		Acceptability Criteria	Method Results	
LLOQ:	%CV	<15%	4.98%	Acceptable
	Accuracy	<20%	11.73%	Acceptable
Low Control:	%CV	<15%	3.24%	Acceptable
	Accuracy	<15%	-1.42%	Acceptable
High Control:	%CV	<15%	3.62%	Acceptable
	Accuracy	<15%	-1.38%	Acceptable
Between Run Precision:				
		Acceptability Criteria	Method Results	
LLOQ:	%CV	<15%	5.65%	Acceptable
	Accuracy	<20%	11.73%	Acceptable
Low Control:	%CV	<15%	5.78%	Acceptable
	Accuracy	<15%	-1.42%	Acceptable
High Control:	%CV	<15%	6.23%	Acceptable
	Accuracy	<15%	-1.38%	Acceptable

Appendix C

Dilution Integrity: Method Bias, Precision and Accuracy				
Bias				
	Acceptability Criteria	Method Results		
1:2- 50 ng/mL	Mean Bias % of 5 Days (<20%)	3.0%		Acceptable
1:10- 50 ng/mL		11.0%		Acceptable
1:50- 50 ng/mL		3.6%		Acceptable
Within Run Precision				
		Acceptability Criteria	Method Results	
1:2- 50 ng/mL	%CV	<15%	3.58%	Acceptable
	Accuracy	<20%	0.46%	Acceptable
1:10- 50 ng/mL	%CV	<15%	6.65%	Acceptable
	Accuracy	<15%	-11.0%	Acceptable
1:50- 50 ng/mL	%CV	<15%	2.69%	Acceptable
	Accuracy	<15%	3.6%	Acceptable
Between Run Precision				
		Acceptability Criteria	Method Results	
1:2- 50 ng/mL	%CV	<15%	6.74%	Acceptable
	Accuracy	<20%	4.0%	Acceptable
1:10- 50 ng/mL	%CV	<15%	8.4%	Acceptable
	Accuracy	<15%	-3.05%	Acceptable
1:50- 50 ng/mL	%CV	<15%	8.86%	Acceptable
	Accuracy	<15%	-9.59%	Acceptable

Appendix C

Validation Data-Dimethylone in Blood

Method Limitations	
Limit of Detection:	5 ng/mL
Limit of Quantitation:	5 ng/mL
Carryover <:	5000 ng/mL

Calibration Curve Information			
	Acceptability Criteria	Method Results	
Slope:	1 (0.95-1.05)	1.0018	Acceptable
r2:	1 (0.98-1.00)	0.998	Acceptable
Intercept:	0 (-0.5-0.5)	-0.002	Acceptable
Linear Dynamic Range:	5-500 ng/mL		
Calibration Range:	5-500 ng/mL		

Percent Recovery			
	Acceptability Criteria	Method Results	
Compound:	>70%	92.04%	Acceptable

Stability of Compound			
Processed Sample Stability			
	Time	Peak Area	Stability
Low Control	24 Hour	3461.66	Unstable
	48 Hour	3336	Unstable
	72 Hour	3490.66	Unstable
High Control	24 Hour	56244.66	Unstable
	48 Hour	60810.66	Unstable
	72 Hour	66138.66	Unstable

Ionization Suppression/Enhancement Percentage				
		Acceptability Criteria	Method Results	
Low Control:	Suppression or Enhancement	±25%	22.88%	Acceptable
	%CV	< 15%	14.73%	Acceptable
High Control:	Suppression or Enhancement	±25%	-1.0%	Acceptable
	%CV	< 15%	10.31%	Acceptable

Appendix C

Matrix Match Acceptance					
Matrix:			Acceptability Criteria	Method Results	
Urine	Low Control (15 ng/mL)	Mean	±20% Target	14.1 (6.0%)	Acceptable
		%CV	<20%	11.01%	Acceptable
	High Control (350 ng/mL)	Mean	±20% Target	320.6 (8.4%)	Acceptable
		%CV	<20%	10.58%	Acceptable

Interferences			
	Interference?		Interference?
Matrix	NO	MDA	NO
Internal Standard with Drug	NO	MDMA	NO
Drug with Internal Standard	NO	MDPV	NO
2-C-T-7	NO	Methadone	NO
3,4 Dimethylmethcathinone	NO	Methamphetamine	NO
Acetaminophen	NO	Morphine	NO
Amphetamine	NO	Oxycodone	NO
Caffeine	NO	Tramadol	NO
Clonazepam	NO	O-Desmethyltramadol	NO
Cocaine	NO	Diazepam	NO
Codeine	NO	MDPP	NO
Cotinine	NO		

Appendix C

Method Bias, Precision and Accuracy				
Bias				
	Acceptability Criteria	Method Results		
LLOQ:	Mean Bias % of 5 Days (<20%)	10.53%		Acceptable
Low Control:		5.24%		Acceptable
High Control:		5.8%		Acceptable
Within Run Precision:				
		Acceptability Criteria	Method Results	
LLOQ:	%CV	<15%	14.36%	Acceptable
	Accuracy	<20%	-3.33%	Acceptable
Low Control:	%CV	<15%	5.49%	Acceptable
	Accuracy	<15%	-3.2%	Acceptable
High Control:	%CV	<15%	3.02%	Acceptable
	Accuracy	<15%	-5.8%	Acceptable
Between Run Precision:				
		Acceptability Criteria	Method Results	
LLOQ:	%CV	<15%	12.58%	Acceptable
	Accuracy	<20%	-3.33%	Acceptable
Low Control:	%CV	<15%	7.20%	Acceptable
	Accuracy	<15%	-3.2%	Acceptable
High Control:	%CV	<15%	5.14%	Acceptable
	Accuracy	<15%	-5.8%	Acceptable

Appendix C

Dilution Integrity: Method Bias, Precision and Accuracy				
Bias				
	Acceptability Criteria	Method Results		
1:2- 50 ng/mL	Mean Bias % of 5 Days (<20%)	3.94%		Acceptable
1:10- 50 ng/mL		13.86%		Acceptable
Within Run Precision				
		Acceptability Criteria	Method Results	
1:2- 50 ng/mL	%CV	<15%	5.06%	Acceptable
	Accuracy	<20%	-1.42%	Acceptable
1:10- 50 ng/mL	%CV	<15%	4.42%	Acceptable
	Accuracy	<15%	-13.86%	Acceptable
Between Run Precision				
		Acceptability Criteria	Method Results	
1:2- 50 ng/mL	%CV	<15%	7.16%	Acceptable
	Accuracy	<20%	-1.42%	Acceptable
1:10- 50 ng/mL	%CV	<15%	7.15%	Acceptable
	Accuracy	<15%	-13.86%	Acceptable

Validation Data-Ethylone in Blood

Method Limitations	
Limit of Detection:	5 ng/mL
Limit of Quantitation:	5 ng/mL
Carryover <:	5000 ng/mL

Calibration Curve Information			
	Acceptability Criteria	Method Results	
Slope:	1 (0.95-1.05)	1.0076	Acceptable
r2:	1 (0.98-1.00)	0.997	Acceptable
Intercept:	0 (-0.5-0.5)	-0.009	Acceptable
Linear Dynamic Range:	5-500 ng/mL		
Calibration Range:	5-500 ng/mL		

Percent Recovery			
	Acceptability Criteria	Method Results	
Compound:	>70%	91.82%	Acceptable

Stability of Compound			
Processed Sample Stability			
	Time	Peak Area	Stability
Low Control	24 Hour	7725	Unstable
	48 Hour	7352.666667	Unstable
	72 Hour	7674.333333	Unstable
High Control	24 Hour	135765.6667	Unstable
	48 Hour	144295.3333	Unstable
	72 Hour	150351.3333	Unstable

Ionization Suppression/Enhancement Percentage				
		Acceptability Criteria	Method Results	
Low Control:	Suppression or Enhancement	±25%	6.97%	Acceptable
	%CV	< 15%	7.52%	Acceptable
High Control:	Suppression or Enhancement	±25%	-9.15%	Acceptable
	%CV	< 15%	5.87%	Acceptable

Appendix C

Matrix Match Acceptance					
Matrix:			Acceptability Criteria	Method Results	
Urine	Low Control (15 ng/mL)	Mean	±20% Target	15.5 (3.33%)	Acceptable
		%CV	<20%	5.65%	Acceptable
	High Control (350 ng/mL)	Mean	±20% Target	347.0 (0.85%)	Acceptable
		%CV	<20%	6.62%	Acceptable

Interferences			
	Interference?		Interference?
Matrix	NO	MDA	NO
Internal Standard with Drug	NO	MDMA	NO
Drug with Internal Standard	NO	MDPV	NO
2-C-T-7	NO	Methadone	NO
3,4 Dimethylmethcathinone	NO	Methamphetamine	NO
Acetaminophen	NO	Morphine	NO
Amphetamine	NO	Oxycodone	NO
Caffeine	NO	Tramadol	NO
Clonazepam	NO	O-Desmethyltramadol	NO
Cocaine	NO	Diazepam	NO
Codeine	NO	MDPP	NO
Cotinine	NO		

Appendix C

Method Bias, Precision and Accuracy				
Bias				
	Acceptability Criteria	Method Results		
LLOQ:	Mean Bias % of 5 Days (<20%)	8.13%		Acceptable
Low Control:		2.84%		Acceptable
High Control:		3.94%		Acceptable
Within Run Precision:				
		Acceptability Criteria	Method Results	
LLOQ:	%CV	<15%	8.67%	Acceptable
	Accuracy	<20%	8.13%	Acceptable
Low Control:	%CV	<15%	3.47%	Acceptable
	Accuracy	<15%	-0.26%	Acceptable
High Control:	%CV	<15%	4.18%	Acceptable
	Accuracy	<15%	-2.52%	Acceptable
Between Run Precision:				
		Acceptability Criteria	Method Results	
LLOQ:	%CV	<15%	9.23%	Acceptable
	Accuracy	<20%	8.13%	Acceptable
Low Control:	%CV	<15%	6.28%	Acceptable
	Accuracy	<15%	-0.26%	Acceptable
High Control:	%CV	<15%	6.59%	Acceptable
	Accuracy	<15%	-2.52%	Acceptable

Appendix C

Dilution Integrity: Method Bias, Precision and Accuracy				
Bias				
	Acceptability Criteria	Method Results		
1:2- 50 ng/mL	Mean Bias % of 5 Days (<20%)	5.98%		Acceptable
1:10- 50 ng/mL		10.37%		Acceptable
1:50- 50 ng/mL		13.13%		Acceptable
Within Run Precision				
		Acceptability Criteria	Method Results	
1:2- 50 ng/mL	%CV	<15%	5.76%	Acceptable
	Accuracy	<20%	4.22%	Acceptable
1:10- 50 ng/mL	%CV	<15%	7.80%	Acceptable
	Accuracy	<15%	-10.37%	Acceptable
1:50- 50 ng/mL	%CV	<15%	5.16%	Acceptable
	Accuracy	<15%	13.13%	Acceptable
Between Run Precision				
		Acceptability Criteria	Method Results	
1:2- 50 ng/mL	%CV	<15%	9.29%	Acceptable
	Accuracy	<20%	12.79%	Acceptable
1:10- 50 ng/mL	%CV	<15%	9.84%	Acceptable
	Accuracy	<15%	2.31%	Acceptable
1:50- 50 ng/mL	%CV	<15%	9.58%	Acceptable
	Accuracy	<15%	-0.93%	Acceptable

Appendix C

Validation Data-4-Fluoroamphetamine in Blood

Method Limitations	
Limit of Detection:	5 ng/mL
Limit of Quantitation:	5 ng/mL
Carryover <:	5000 ng/mL

Calibration Curve Information			
	Acceptability Criteria	Method Results	
Slope:	1 (0.95-1.05)	1.0173	Acceptable
r2:	1 (0.98-1.00)	0.997	Acceptable
Intercept:	0 (-0.5-0.5)	-0.006	Acceptable
Linear Dynamic Range:	5-500 ng/mL		
Calibration Range:	5-500 ng/mL		

Percent Recovery			
	Acceptability Criteria	Method Results	
Compound:	>70%	87.29%	Acceptable

Stability of Compound			
Processed Sample Stability			
	Time	Peak Area	Stability
Low Control	24 Hour	2392.33	Stable
	48 Hour	2389.66	Stable
	72 Hour	2391	Stable
High Control	24 Hour	42276	Stable
	48 Hour	45382.33	Unstable
	72 Hour	45872.33	Unstable

Ionization Suppression/Enhancement Percentage				
		Acceptability Criteria	Method Results	
Low Control:	Suppression or Enhancement	±25%	59.31%	Unacceptable
	%CV	< 15%	11.30%	Acceptable
High Control:	Suppression or Enhancement	±25%	11.21%	Acceptable
	%CV	< 15%	4.36%	Acceptable

Appendix C

Matrix Match Acceptance					
Matrix:			Acceptability Criteria	Method Results	
Urine	Low Control (15 ng/mL)	Mean	±20% Target	13.0 (13.3%)	Acceptable
		%CV	<20%	4.92%	Acceptable
	High Control (350 ng/mL)	Mean	±20% Target	312.8 (10.6%)	Acceptable
		%CV	<20%	6.04%	Acceptable

Interferences			
	Interference?		Interference?
Matrix	NO	MDA	NO
Internal Standard with Drug	NO	MDMA	NO
Drug with Internal Standard	NO	MDPV	NO
2-C-T-7	NO	Methadone	NO
3,4 Dimethylmethcathinone	NO	Methamphetamine	NO
Acetaminophen	NO	Morphine	NO
Amphetamine	NO	Oxycodone	NO
Caffeine	NO	Tramadol	NO
Clonazepam	NO	O-Desmethyltramadol	NO
Cocaine	NO	Diazepam	NO
Codeine	NO	MDPP	NO
Cotinine	NO		

Appendix C

Method Bias, Precision and Accuracy				
Bias				
	Acceptability Criteria	Method Results		
LLOQ:	Mean Bias % of 5 Days (<20%)	16.66%		Acceptable
Low Control:		4.57%		Acceptable
High Control:		4.08%		Acceptable
Within Run Precision:				
		Acceptability Criteria	Method Results	
LLOQ:	%CV	<15%	9.19%	Acceptable
	Accuracy	<20%	14.8%	Acceptable
Low Control:	%CV	<15%	5.55%	Acceptable
	Accuracy	<15%	0.84%	Acceptable
High Control:	%CV	<15%	4.18%	Acceptable
	Accuracy	<15%	-4.03%	Acceptable
Between Run Precision:				
		Acceptability Criteria	Method Results	
LLOQ:	%CV	<15%	11.29%	Acceptable
	Accuracy	<20%	14.28%	Acceptable
Low Control:	%CV	<15%	7.77%	Acceptable
	Accuracy	<15%	0.84%	Acceptable
High Control:	%CV	<15%	6.27%	Acceptable
	Accuracy	<15%	-4.03%	Acceptable

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Dilution Integrity: Method Bias, Precision and Accuracy				
Bias				
	Acceptability Criteria	Method Results		
1:2- 50 ng/mL	Mean Bias % of 5 Days (<20%)	8.73%		Acceptable
1:10- 50 ng/mL		19.46%		Acceptable
1:50- 50 ng/mL		11.61%		Acceptable
Within Run Precision				
		Acceptability Criteria	Method Results	
1:2- 50 ng/mL	%CV	<15%	9.21%	Acceptable
	Accuracy	<20%	-5.4%	Acceptable
1:10- 50 ng/mL	%CV	<15%	2.37%	Acceptable
	Accuracy	<15%	-19.46%	Acceptable
1:50- 50 ng/mL	%CV	<15%	12.51%	Acceptable
	Accuracy	<15%	-10.57%	Acceptable
Between Run Precision				
		Acceptability Criteria	Method Results	
1:2- 50 ng/mL	%CV	<15%	11.64%	Acceptable
	Accuracy	<20%	-5.4%	Acceptable
1:10- 50 ng/mL	%CV	<15%	5.17%	Acceptable
	Accuracy	<15%	-19.72%	Acceptable
1:50- 50 ng/mL	%CV	<15%	14.76%	Acceptable
	Accuracy	<15%	-10.57%	Acceptable

Validation Data-Butylone in Blood

Method Limitations	
Limit of Detection:	5 ng/mL
Limit of Quantitation:	5 ng/mL
Carryover <:	5000 ng/mL

Calibration Curve Information			
	Acceptability Criteria	Method Results	
Slope:	1 (0.95-1.05)	1.0112	Acceptable
r2:	1 (0.98-1.00)	0.995	Acceptable
Intercept:	0 (-0.5-0.5)	0.001	Acceptable
Linear Dynamic Range:	5-500 ng/mL		
Calibration Range:	5-500 ng/mL		

Percent Recovery			
	Acceptability Criteria	Method Results	
Compound:	>70%	93.63%	Acceptable

Stability of Compound			
Processed Sample Stability			
	Time	Peak Area	Stability
Low Control	24 Hour	1672.333333	Stable
	48 Hour	1904.333333	Unstable
	72 Hour	1979.333333	Unstable
High Control	24 Hour	30327.66667	Unstable
	48 Hour	32525.66667	Unstable
	72 Hour	34697	Unstable

Ionization Suppression/Enhancement Percentage				
		Acceptability Criteria	Method Results	
Low Control:	Suppression or Enhancement	±25%	9.06%	Acceptable
	%CV	< 15%	7.97%	Acceptable
High Control:	Suppression or Enhancement	±25%	-5.05%	Acceptable
	%CV	< 15%	7.42%	Acceptable

Appendix C

Matrix Match Acceptance					
Matrix:			Acceptability Criteria	Method Results	
Urine	Low Control (15 ng/mL)	Mean	±20% Target	12.8 (14.6%)	Acceptable
		%CV	<20%	12.5%	Acceptable
	High Control (350 ng/mL)	Mean	±20% Target	310.6 (11.2%)	Acceptable
		%CV	<20%	8.39%	Acceptable

Interferences			
	Interference?		Interference?
Matrix	NO	MDA	NO
Internal Standard with Drug	NO	MDMA	NO
Drug with Internal Standard	NO	MDPV	NO
2-C-T-7	NO	Methadone	NO
3,4 Dimethylmethcathinone	NO	Methamphetamine	NO
Acetaminophen	NO	Morphine	NO
Amphetamine	NO	Oxycodone	NO
Caffeine	NO	Tramadol	NO
Clonazepam	NO	O-Desmethyltramadol	NO
Cocaine	NO	Diazepam	NO
Codeine	NO	MDPP	NO
Cotinine	NO		

Appendix C

Method Bias, Precision and Accuracy				
Bias				
	Acceptability Criteria	Method Results		
LLOQ:	Mean Bias % of 5 Days (<20%)	9.89%		Acceptable
Low Control:		4.23%		Acceptable
High Control:		3.78%		Acceptable
Within Run Precision:				
		Acceptability Criteria	Method Results	
LLOQ:	%CV	<15%	8.22%	Acceptable
	Accuracy	<20%	7.76%	Acceptable
Low Control:	%CV	<15%	3.82%	Acceptable
	Accuracy	<15%	3.70%	Acceptable
High Control:	%CV	<15%	3.10%	Acceptable
	Accuracy	<15%	-3.78%	Acceptable
Between Run Precision:				
		Acceptability Criteria	Method Results	
LLOQ:	%CV	<15%	10.73%	Acceptable
	Accuracy	<20%	7.76%	Acceptable
Low Control:	%CV	<15%	7.33%	Acceptable
	Accuracy	<15%	3.70%	Acceptable
High Control:	%CV	<15%	5.02%	Acceptable
	Accuracy	<15%	-3.78%	Acceptable

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Dilution Integrity: Method Bias, Precision and Accuracy				
Bias				
	Acceptability Criteria	Method Results		
1:2- 50 ng/mL	Mean Bias % of 5 Days (<20%)	3.73%		Acceptable
1:10- 50 ng/mL		10.24%		Acceptable
1:50- 50 ng/mL		9.05%		Acceptable
Within Run Precision				
		Acceptability Criteria	Method Results	
1:2- 50 ng/mL	%CV	<15%	4.96%	Acceptable
	Accuracy	<20%	1.78%	Acceptable
1:10- 50 ng/mL	%CV	<15%	6.76%	Acceptable
	Accuracy	<15%	-10.24%	Acceptable
1:50- 50 ng/mL	%CV	<15%	9.58%	Acceptable
	Accuracy	<15%	-7.66%	Acceptable
Between Run Precision				
		Acceptability Criteria	Method Results	
1:2- 50 ng/mL	%CV	<15%	8.18%	Acceptable
	Accuracy	<20%	1.78%	Acceptable
1:10- 50 ng/mL	%CV	<15%	9.85%	Acceptable
	Accuracy	<15%	-10.24%	Acceptable
1:50- 50 ng/mL	%CV	<15%	11.86%	Acceptable
	Accuracy	<15%	-7.66%	Acceptable

Validation Data-Alpha-PVP in Blood

Method Limitations	
Limit of Detection:	5 ng/mL
Limit of Quantitation:	5 ng/mL
Carryover <:	5000 ng/mL

Calibration Curve Information			
	Acceptability Criteria	Method Results	
Slope:	1 (0.95-1.05)	1.015	Acceptable
r2:	1 (0.98-1.00)	0.998	Acceptable
Intercept:	0 (-0.5-0.5)	0.005	Acceptable
Linear Dynamic Range:	5-500 ng/mL		
Calibration Range:	5-500 ng/mL		

Percent Recovery			
	Acceptability Criteria	Method Results	
Compound:	>70%	92.31%	Acceptable

Stability of Compound			
Processed Sample Stability			
	Time	Peak Area	Stability
Low Control	24 Hour	2941.66	Stable
	48 Hour	2992.33	Stable
	72 Hour	3176	Stable
High Control	24 Hour	57253.33	Stable
	48 Hour	61530.33	Stable
	72 Hour	69529.66	Stable

Ionization Suppression/Enhancement Percentage				
		Acceptability Criteria	Method Results	
Low Control:	Suppression or Enhancement	±25%	14.28%	Acceptable
	%CV	< 15%	3.96%	Acceptable
High Control:	Suppression or Enhancement	±25%	3.35%	Acceptable
	%CV	< 15%	4.43%	Acceptable

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Matrix Match Acceptance					
Matrix:			Acceptability Criteria	Method Results	
Urine	Low Control (15 ng/mL)	Mean	±20% Target	14.5 (3.33%)	Acceptable
		%CV	<20%	1.84%	Acceptable
	High Control (350 ng/mL)	Mean	±20% Target	340.4 (2.68%)	Acceptable
		%CV	<20%	2.21%	Acceptable

Interferences			
	Interference?		Interference?
Matrix	NO	MDA	NO
Internal Standard with Drug	NO	MDMA	NO
Drug with Internal Standard	NO	MDPV	NO
2-C-T-7	NO	Methadone	NO
3,4 Dimethylmethcathinone	NO	Methamphetamine	NO
Acetaminophen	NO	Morphine	NO
Amphetamine	NO	Oxycodone	NO
Caffeine	NO	Tramadol	NO
Clonazepam	NO	O-Desmethyltramadol	NO
Cocaine	NO	Diazepam	NO
Codeine	NO	MDPP	NO
Cotinine	NO		

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Method Bias, Precision and Accuracy				
Bias				
	Acceptability Criteria	Method Results		
LLOQ:	Mean Bias % of 5 Days (<20%)	8.8%		Acceptable
Low Control:		2.97%		Acceptable
High Control:		1.75%		Acceptable
Within Run Precision:				
		Acceptability Criteria	Method Results	
LLOQ:	%CV	<15%	5.31%	Acceptable
	Accuracy	<20%	8.0%	Acceptable
Low Control:	%CV	<15%	3.43%	Acceptable
	Accuracy	<15%	1.91%	Acceptable
High Control:	%CV	<15%	2.06%	Acceptable
	Accuracy	<15%	-0.86%	Acceptable
Between Run Precision:				
		Acceptability Criteria	Method Results	
LLOQ:	%CV	<15%	6.37%	Acceptable
	Accuracy	<20%	8.0%	Acceptable
Low Control:	%CV	<15%	4.44%	Acceptable
	Accuracy	<15%	1.91%	Acceptable
High Control:	%CV	<15%	2.67%	Acceptable
	Accuracy	<15%	-0.86%	Acceptable

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Dilution Integrity: Method Bias, Precision and Accuracy				
Bias				
	Acceptability Criteria	Method Results		
1:2- 50 ng/mL	Mean Bias % of 5 Days (<20%)	3.16%		Acceptable
1:10- 50 ng/mL		13.45%		Acceptable
1:50- 50 ng/mL		3.48%		Acceptable
Within Run Precision				
		Acceptability Criteria	Method Results	
1:2- 50 ng/mL	%CV	<15%	3.38%	Acceptable
	Accuracy	<20%	-2.57%	Acceptable
1:10- 50 ng/mL	%CV	<15%	6.69%	Acceptable
	Accuracy	<15%	-13.45%	Acceptable
1:50- 50 ng/mL	%CV	<15%	4.76%	Acceptable
	Accuracy	<15%	1.88%	Acceptable
Between Run Precision				
		Acceptability Criteria	Method Results	
1:2- 50 ng/mL	%CV	<15%	5.93%	Acceptable
	Accuracy	<20%	-2.57%	Acceptable
1:10- 50 ng/mL	%CV	<15%	8.36%	Acceptable
	Accuracy	<15%	-13.45%	Acceptable
1:50- 50 ng/mL	%CV	<15%	10.0%	Acceptable
	Accuracy	<15%	1.88%	Acceptable

Validation Data-Methylone in Oral Fluid

Method Limitations	
Limit of Detection:	5 ng/mL
Limit of Quantitation:	5 ng/mL
Carryover <:	5000 ng/mL

Calibration Curve Information			
	Acceptability Criteria	Method Results	
Slope:	1 (0.95-1.05)	0.9994	Acceptable
r2:	1 (0.98-1.00)	0.998	Acceptable
Intercept:	0 (-0.5-0.5)	0.3596	Acceptable
Linear Dynamic Range:	5-500 ng/mL		
Calibration Range:	5-500 ng/mL		

Percent Recovery			
	Acceptability Criteria	Method Results	
Compound:	>70%	91.47%	Acceptable

Interferences			
	Interference?		Interference?
Matrix	NO	MDA	NO
Internal Standard with Drug	NO	MDMA	NO
Drug with Internal Standard	NO	MDPV	NO
2-C-T-7	NO	Methadone	NO
3,4 Dimethylmethcathinone	NO	Methamphetamine	NO
Acetaminophen	NO	Morphine	NO
Amphetamine	NO	Oxycodone	NO
Caffeine	NO	Tramadol	NO
Clonazepam	NO	O-Desmethyltramadol	NO
Cocaine	NO	Diazepam	NO
Codeine	NO	MDPP	NO
Cotinine	NO		

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Method Bias, Precision and Accuracy				
Bias				
	Acceptability Criteria	Method Results		
LLOQ:	Mean Bias % of 5 Days (<20%)	11.76%		Acceptable
Low Control:		8.34%		Acceptable
High Control:		3.29%		Acceptable
Within Run Precision:				
		Acceptability Criteria	Method Results	
LLOQ:	%CV	<15%	14.36%	Acceptable
	Accuracy	<20%	4.0%	Acceptable
Low Control:	%CV	<15%	9.95%	Acceptable
	Accuracy	<15%	2.50%	Acceptable
High Control:	%CV	<15%	4.19%	Acceptable
	Accuracy	<15%	1.30%	Acceptable
Between Run Precision:				
		Acceptability Criteria	Method Results	
LLOQ:	%CV	<15%	14.19%	Acceptable
	Accuracy	<20%	1.82%	Acceptable
Low Control:	%CV	<15%	11.69%	Acceptable
	Accuracy	<15%	2.50%	Acceptable
High Control:	%CV	<15%	5.21%	Acceptable
	Accuracy	<15%	1.30%	Acceptable

Validation Data-Dimethylone in Oral Fluid

Method Limitations	
Limit of Detection:	5 ng/mL
Limit of Quantitation:	5 ng/mL
Carryover <:	5000 ng/mL

Calibration Curve Information			
	Acceptability Criteria	Method Results	
Slope:	1 (0.95-1.05)	1.0021	Acceptable
r2:	1 (0.98-1.00)	0.9956	Acceptable
Intercept:	0 (-0.5-0.5)	0.426	Acceptable
Linear Dynamic Range:	5-500 ng/mL		
Calibration Range:	5-500 ng/mL		

Percent Recovery			
	Acceptability Criteria	Method Results	
Compound:	>70%	111%	Acceptable

Interferences			
	Interference?		Interference?
Matrix	NO	MDA	NO
Internal Standard with Drug	NO	MDMA	NO
Drug with Internal Standard	NO	MDPV	NO
2-C-T-7	NO	Methadone	NO
3,4 Dimethylmethcathinone	NO	Methamphetamine	NO
Acetaminophen	NO	Morphine	NO
Amphetamine	NO	Oxycodone	NO
Caffeine	NO	Tramadol	NO
Clonazepam	NO	O-Desmethyltramadol	NO
Cocaine	NO	Diazepam	NO
Codeine	NO	MDPP	NO
Cotinine	NO		

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Method Bias, Precision and Accuracy				
Bias				
	Acceptability Criteria	Method Results		
LLOQ:	Mean Bias % of 5 Days (<20%)	19.9%		Acceptable
Low Control:		12.05%		Acceptable
High Control:		4.36%		Acceptable
Within Run Precision:				
		Acceptability Criteria	Method Results	
LLOQ*:	%CV	<15%	17.69%	Acceptable
	Accuracy	<20%	15.4%	Acceptable
Low Control:	%CV	<15%	11.0%	Acceptable
	Accuracy	<15%	8.57%	Acceptable
High Control:	%CV	<15%	5.64%	Acceptable
	Accuracy	<15%	3.32%	Acceptable
Between Run Precision:				
		Acceptability Criteria	Method Results	
LLOQ*:	%CV	<15%	18.44%	Acceptable
	Accuracy	<20%	15.4%	Acceptable
Low Control:	%CV	<15%	12.03%	Acceptable
	Accuracy	<15%	8.57%	Acceptable
High Control:	%CV	<15%	6.37%	Acceptable
	Accuracy	<15%	3.32%	Acceptable

*%CV≤20% was determined to be acceptable

Validation Data-Ethylone in Oral Fluid

Method Limitations	
Limit of Detection:	5 ng/mL
Limit of Quantitation:	5 ng/mL
Carryover <:	5000 ng/mL

Calibration Curve Information			
	Acceptability Criteria	Method Results	
Slope:	1 (0.95-1.05)	1.0009	Acceptable
r2:	1 (0.98-1.00)	0.9977	Acceptable
Intercept:	0 (-0.5-0.5)	0.495	Acceptable
Linear Dynamic Range:	5-500 ng/mL		
Calibration Range:	5-500 ng/mL		

Percent Recovery			
	Acceptability Criteria	Method Results	
Compound:	>70%	96.9%	Acceptable

Interferences			
	Interference?		Interference?
Matrix	NO	MDA	NO
Internal Standard with Drug	NO	MDMA	NO
Drug with Internal Standard	NO	MDPV	NO
2-C-T-7	NO	Methadone	NO
3,4 Dimethylmethcathinone	NO	Methamphetamine	NO
Acetaminophen	NO	Morphine	NO
Amphetamine	NO	Oxycodone	NO
Caffeine	NO	Tramadol	NO
Clonazepam	NO	O-Desmethyltramadol	NO
Cocaine	NO	Diazepam	NO
Codeine	NO	MDPP	NO
Cotinine	NO		

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Method Bias, Precision and Accuracy				
Bias				
	Acceptability Criteria	Method Results		
LLOQ:	Mean Bias % of 5 Days (<20%)	7.06%		Acceptable
Low Control:		10.33%		Acceptable
High Control:		8.86%		Acceptable
Within Run Precision:				
		Acceptability Criteria	Method Results	
LLOQ:	%CV	<15%	8.86%	Acceptable
	Accuracy	<20%	3.8%	Acceptable
Low Control:	%CV	<15%	7.90%	Acceptable
	Accuracy	<15%	7.57%	Acceptable
High Control:	%CV	<15%	6.69%	Acceptable
	Accuracy	<15%	8.86%	Acceptable
Between Run Precision:				
		Acceptability Criteria	Method Results	
LLOQ:	%CV	<15%	12.46%	Acceptable
	Accuracy	<20%	3.8%	Acceptable
Low Control:	%CV	<15%	9.22%	Acceptable
	Accuracy	<15%	7.57%	Acceptable
High Control:	%CV	<15%	6.96%	Acceptable
	Accuracy	<15%	8.86%	Acceptable

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Validation Data-4-Fluoroamphetamine in Oral Fluid

Method Limitations	
Limit of Detection:	5 ng/mL
Limit of Quantitation:	5 ng/mL
Carryover <:	5000 ng/mL

Calibration Curve Information			
	Acceptability Criteria	Method Results	
Slope:	1 (0.95-1.05)	1.0009	Acceptable
r2:	1 (0.98-1.00)	0.9976	Acceptable
Intercept:	0 (-0.5-0.5)	0.055	Acceptable
Linear Dynamic Range:	5-500 ng/mL		
Calibration Range:	5-500 ng/mL		

Percent Recovery			
	Acceptability Criteria	Method Results	
Compound:	>70%	103.4%	Acceptable

Interferences			
	Interference?		Interference?
Matrix	NO	MDA	NO
Internal Standard with Drug	NO	MDMA	NO
Drug with Internal Standard	NO	MDPV	NO
2-C-T-7	NO	Methadone	NO
3,4 Dimethylmethcathinone	NO	Methamphetamine	NO
Acetaminophen	NO	Morphine	NO
Amphetamine	NO	Oxycodone	NO
Caffeine	NO	Tramadol	NO
Clonazepam	NO	O-Desmethyltramadol	NO
Cocaine	NO	Diazepam	NO
Codeine	NO	MDPP	NO
Cotinine	NO		

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Method Bias, Precision and Accuracy				
Bias				
	Acceptability Criteria	Method Results		
LLOQ:	Mean Bias % of 5 Days (<20%)	18.43%		Acceptable
Low Control:		17.87%		Acceptable
High Control:		8.68%		Acceptable
Within Run Precision:				
		Acceptability Criteria	Method Results	
LLOQ:	%CV	<15%	14.46%	Acceptable
	Accuracy	<20%	-10.63%	Acceptable
Low Control*:	%CV	<15%	20.0%	Acceptable
	Accuracy	<15%	0.73%	Acceptable
High Control:	%CV	<15%	10.38%	Acceptable
	Accuracy	<15%	-4.28%	Acceptable
Between Run Precision:				
		Acceptability Criteria	Method Results	
LLOQ*:	%CV	<15%	18.59%	Acceptable
	Accuracy	<20%	-13.5%	Acceptable
Low Control*:	%CV	<15%	19.61%	Acceptable
	Accuracy	<15%	-2.3%	Acceptable
High Control:	%CV	<15%	11.93%	Acceptable
	Accuracy	<15%	-4.28%	Acceptable

*%CV≤20% was determined to be acceptable

Appendix C

Validation Data-Butylone in Oral Fluid

Method Limitations	
Limit of Detection:	5 ng/mL
Limit of Quantitation:	5 ng/mL
Carryover <:	5000 ng/mL

Calibration Curve Information			
	Acceptability Criteria	Method Results	
Slope:	1 (0.95-1.05)	1.0007	Acceptable
r2:	1 (0.98-1.00)	0.9965	Acceptable
Intercept:	0 (-0.5-0.5)	0.4248	Acceptable
Linear Dynamic Range:	5-500 ng/mL		
Calibration Range:	5-500 ng/mL		

Percent Recovery			
	Acceptability Criteria	Method Results	
Compound:	>70%	98.32%	Acceptable

Interferences			
	Interference?		Interference?
Matrix	NO	MDA	NO
Internal Standard with Drug	NO	MDMA	NO
Drug with Internal Standard	NO	MDPV	NO
2-C-T-7	NO	Methadone	NO
3,4 Dimethylmethcathinone	NO	Methamphetamine	NO
Acetaminophen	NO	Morphine	NO
Amphetamine	NO	Oxycodone	NO
Caffeine	NO	Tramadol	NO
Clonazepam	NO	O-Desmethyltramadol	NO
Cocaine	NO	Diazepam	NO
Codeine	NO	MDPP	NO
Cotinine	NO		

Appendix C

Method Bias, Precision and Accuracy				
Bias				
	Acceptability Criteria	Method Results		
LLOQ:	Mean Bias % of 5 Days (<20%)	15.81%		Acceptable
Low Control:		6.44%		Acceptable
High Control:		18.23%		Acceptable
Within Run Precision:				
		Acceptability Criteria	Method Results	
LLOQ*:	%CV	<15%	18.96%	Acceptable
	Accuracy	<20%	-13.21%	Acceptable
Low Control:	%CV	<15%	8.83%	Acceptable
	Accuracy	<15%	1.2%	Acceptable
High Control:	%CV	<15%	5.33%	Acceptable
	Accuracy	<15%	10.95%	Acceptable
Between Run Precision:				
		Acceptability Criteria	Method Results	
LLOQ*:	%CV	<15%	19.67%	Acceptable
	Accuracy	<20%	-10.83%	Acceptable
Low Control*:	%CV	<15%	19.01%	Acceptable
	Accuracy	<15%	1.2%	Acceptable
High Control:	%CV	<15%	6.58%	Acceptable
	Accuracy	<15%	10.95%	Acceptable

*%CV≤20% was determined to be acceptable

Appendix C

Validation Data-Alpha-PVP in Oral Fluid

Method Limitations	
Limit of Detection:	5 ng/mL
Limit of Quantitation:	5 ng/mL
Carryover <:	5000 ng/mL

Calibration Curve Information			
	Acceptability Criteria	Method Results	
Slope:	1 (0.95-1.05)	0.9997	Acceptable
r2:	1 (0.98-1.00)	0.9995	Acceptable
Intercept:	0 (-0.5-0.5)	0.2023	Acceptable
Linear Dynamic Range:	5-500 ng/mL		
Calibration Range:	5-500 ng/mL		

Percent Recovery			
	Acceptability Criteria	Method Results	
Compound:	>70%	94.23%	Acceptable

Interferences			
	Interference?		Interference?
Matrix	NO	MDA	NO
Internal Standard with Drug	NO	MDMA	NO
Drug with Internal Standard	NO	MDPV	NO
2-C-T-7	NO	Methadone	NO
3,4 Dimethylmethcathinone	NO	Methamphetamine	NO
Acetaminophen	NO	Morphine	NO
Amphetamine	NO	Oxycodone	NO
Caffeine	NO	Tramadol	NO
Clonazepam	NO	O-Desmethyltramadol	NO
Cocaine	NO	Diazepam	NO
Codeine	NO	MDPP	NO
Cotinine	NO		

Appendix C

Method Bias, Precision and Accuracy				
Bias				
	Acceptability Criteria	Method Results		
LLOQ:	Mean Bias % of 5 Days (<20%)	3.83%		Acceptable
Low Control:		4.79%		Acceptable
High Control:		3.18%		Acceptable
Within Run Precision:				
		Acceptability Criteria	Method Results	
LLOQ:	%CV	<15%	4.07%	Acceptable
	Accuracy	<20%	2.36%	Acceptable
Low Control*:	%CV	<15%	3.10%	Acceptable
	Accuracy	<15%	-1.64%	Acceptable
High Control:	%CV	<15%	1.53%	Acceptable
	Accuracy	<15%	3.6%	Acceptable
Between Run Precision:				
		Acceptability Criteria	Method Results	
LLOQ*:	%CV	<15%	7.06%	Acceptable
	Accuracy	<20%	2.36%	Acceptable
Low Control*:	%CV	<15%	3.93%	Acceptable
	Accuracy	<15%	-1.64%	Acceptable
High Control:	%CV	<15%	2.59%	Acceptable
	Accuracy	<15%	3.6%	Acceptable

Validation Data-THC in Oral Fluid

Method Limitations	
Limit of Detection:	1 ng/mL
Limit of Quantitation:	2 ng/mL
Carryover <:	1000 ng/mL

Calibration Curve Information			
	Acceptability Criteria	Method Results	
Slope:	1 (0.95-1.05)	1.000	Acceptable
r2:	1 (0.98-1.00)	0.999	Acceptable
Intercept:	0 (-0.5-0.5)	-0.04	Acceptable
Linear Dynamic Range:	2-1000 ng/mL		
Calibration Range:	2-1000 ng/mL		

Percent Recovery			
	Acceptability Criteria	Method Results	
Compound:	>70%	48.41%	Acceptable

Ionization Suppression/Enhancement Percentage				
		Acceptability Criteria	Method Results	
Low Control:	Suppression or Enhancement	±25%	-86.67%	Unacceptable
	%CV	< 15%	5.96%	Acceptable

There was possible ion suppression of the analyte, however, this was compensated by the use of an internal standard.

Appendix C

Interferences			
	Interference?		Interference?
Matrix	NO	MDA	NO
Internal Standard with Drug	NO	MDMA	NO
Drug with Internal Standard	NO	MDPV	NO
2-C-T-7	NO	Methadone	NO
3,4 Dimethylmethcathinone	NO	Methamphetamine	NO
Acetaminophen	NO	Morphine	NO
Amphetamine	NO	Oxycodone	NO
Caffeine	NO	Tramadol	NO
Clonazepam	NO	O-Desmethyltramadol	NO
Cocaine	NO	Diazepam	NO
Codeine	NO	MDPP	NO
Cotinine	NO		

Appendix C

Method Bias, Precision and Accuracy				
Bias				
	Acceptability Criteria	Method Results		
LLOQ:	Mean Bias % of 5 Days (<20%)	7.33%		Acceptable
Low Control:		6.67%		Acceptable
High Control:		3.37%		Acceptable
Within Run Precision:				
		Acceptability Criteria	Method Results	
LLOQ:	%CV	<15%	7.89%	Acceptable
	Accuracy	<20%	-6.00%	Acceptable
Low Control:	%CV	<15%	5.24%	Acceptable
	Accuracy	<20%	-6.00%	Acceptable
High Control:	%CV	<15%	2.88%	Acceptable
	Accuracy	<20%	-0.17%	Acceptable
Between Run Precision:				
		Acceptability Criteria	Method Results	
LLOQ:	%CV	<15%	12.58%	Acceptable
	Accuracy	>80%	-6.00%	Acceptable
Low Control:	%CV	<15%	5.55%	Acceptable
	Accuracy	>80%	-6.00%	Acceptable
High Control:	%CV	<15%	2.90%	Acceptable
	Accuracy	>80%	-0.17%	Acceptable

Appendix C

Dilution Integrity: Method Bias, Precision and Accuracy				
Bias				
	Acceptability Criteria	Method Results		
1:2- 100 ng/mL	Mean Bias % of 5 Days (<20%)	4.94%		Acceptable
1:10- 100 ng/mL		8.29%		Acceptable
Within Run Precision				
		Acceptability Criteria	Method Results	
1:2- 100 ng/mL	%CV	<15%	2.45%	Acceptable
	Accuracy	<15%	-4.94%	Acceptable
1:10- 100 ng/mL	%CV	<15%	3.68%	Acceptable
	Accuracy	<15%	-8.29%	Acceptable
Between Run Precision				
		Acceptability Criteria	Method Results	
1:2- 50 ng/mL	%CV	<15%	3.54%	Acceptable
	Accuracy	<15%	-4.94%	Acceptable
1:10- 50 ng/mL	%CV	<15%	5.57%	Acceptable
	Accuracy	<15%	-8.29%	Acceptable

Appendix C

QTOF Verification-Blood

Verification Subset Sample A - Blood

Component Name	Conc (ng/mL)	Within Run ppm Error	Between Run ppm Error	Within Run RT Error	Between Run RT Error	Average Response
6-Monoacetylmorphine	10	<5	<5	<±0.25	<±0.25	19,261
7-Amino Flunitrazepam	10	<5	<5	<±0.25	<±0.25	17,665
Acetaminophen	20,000	<5	<5	<±0.25	<±0.25	61,833
Alpha-Hydroxyalprazolam	20	<5	<5	<±0.25	<±0.25	1,828
Amitriptyline	50	<5	<5	<±0.25	<±0.25	200,304
Amoxapine	50	<5	<5	<±0.25	<±0.25	75,582
Atomoxetine	100	<5	<5	<±0.25	<±0.25	107,040
Atropine	1,000	>5 (2/9)	>5 (2/9)	<±0.25	<±0.25	794,494
Bromo-Dragon FLY	10	<5	<5	>±0.25	>±0.25	4,202
Cephaeline	5	<5	<5	<±0.25	<±0.25	9,270
Chlorpromazine	20	<5	<5	<±0.25	<±0.25	51,192
Clomipramine	50	<5	<5	<±0.25	<±0.25	145,239
Clozapine	50	<5	<5	<±0.25	<±0.25	160,930
Cocaethylene	20	<5	<5	<±0.25	<±0.25	97,403
Desipramine	50	<5	<5	>±0.25	>±0.25	154,028
Dextrorphan / Levorphanol	100	<5	<5	<±0.25	<±0.25	235,682
Dihydrocodeine / Hydrocodol	10	<5	<5	<±0.25	<±0.25	25,698
Diltiazem	100	<5	<5	<±0.25	<±0.25	518,859
Doxylamine	50	<5	<5	<±0.25	<±0.25	24,890
EDDP	50	<5	<5	<±0.25	<±0.25	106,403

Estazolam	20	<5	<5	<±0.25	<±0.25	10,500
Eszopiclone / Zopiclone	10	<5	<5	<±0.25	<±0.25	2,359
Etodolac	50,000	<5	<5	<±0.25	<±0.25	3,157
Flunitrazepam	5	<5	<5	<±0.25	<±0.25	5,215
Fluoxetine	50	<5	<5	<±0.25	<±0.25	133,973
Fluphenazine	5	<5	<5	<±0.25	<±0.25	16,434
Fluvoxamine	250	<5	<5	<±0.25	<±0.25	220,131
Hydroxybupropion	100	<5	<5	<±0.25	<±0.25	61,108
Hydroxyethylflurazepam	25	<5	<5	<±0.25	<±0.25	10,905
Imipramine	25	<5	<5	<±0.25	<±0.25	100,818
Indomethacin	5,000	MT (6/9)	MT (6/9)	MT (6/9)	MT (6/9)	556
Lacosamide	10	<5	<5	<±0.25	<±0.25	1,588
Laudanosine	10	<5	<5	<±0.25	<±0.25	1,978
Levetiracetam	5,000	MT	MT	MT	MT	MT
Lidocaine	1,000	<5	<5	<±0.25	<±0.25	606,592
Loxapine	50	<5	<5	<±0.25	<±0.25	185,937
MDA	10	MT (1/9)	MT (1/9)	MT (1/9)	MT (1/9)	412
MDEA	10	<5	<5	<±0.25	<±0.25	4,548
Meprobamate	1,000	<5	<5	<±0.25	<±0.25	1,026
Mescaline	10	MT	MT	MT	MT	MT
Mesoridazine	100	<5	<5	<±0.25	<±0.25	412,091
Methaqualone	5,000	<5	<5	<±0.25	<±0.25	999,475
Methcathinone	10	<5	<5	<±0.25	<±0.25	3,960
Methylphenidate	10	<5	<5	<±0.25	<±0.25	25,604
Metoclopramide	10	<5	<5	<±0.25	<±0.25	26,347

Mitragynine	10	<5	<5	<±0.25	<±0.25	58,906
Morphine	10	<5	<5	<±0.25	<±0.25	2,219
Naltrexone	1	<5	<5	<±0.25	<±0.25	1,626
Naproxen	500,000	<5	<5	<±0.25	<±0.25	2,696
Nicotine	100	<5	<5	<±0.25	<±0.25	20,871
Norbuprenorphine	2	<5	<5	<±0.25	<±0.25	3,134
Nordiazepam	25	<5	<5	<±0.25	<±0.25	6,999
Norfentanyl	1	MT (1/9)	MT (1/9)	MT (1/9)	MT (1/9)	634
Norfluoxetine	100	<5	<5	<±0.25	<±0.25	4,553
Norketamine	20	<5	<5	<±0.25	<±0.25	2,508
Norpropoxyphene	250	<5	<5	<±0.25	<±0.25	17,689
O-Desmethyltramadol	25	<5	<5	<±0.25	<±0.25	48,590
Oxazepam	50	<5	<5	<±0.25	<±0.25	4,057
Oxymorphone	10	<5	<5	<±0.25	<±0.25	2,869
Papaverine	500	<5	<5	<±0.25	<±0.25	1,164,256
Phencyclidine	5	<5	<5	<±0.25	<±0.25	2,522
Pheniramine	10	<5	<5	<±0.25	<±0.25	11,088
PMA	10	MT	MT	MT	MT	MT
Quetiapine	100	<5	<5	<±0.25	<±0.25	479,465
Quinine	2,000	>5 (1X)	>5 (1X)	<±0.25	<±0.25	1,288,434
Risperidone	5	<5	<5	<±0.25	<±0.25	24,092
Sildenafil	50	<5	<5	<±0.25	<±0.25	46,248
Tapentadol	10	<5	<5	>±0.25	>±0.25	18,966
Temazepam	50	<5	<5	<±0.25	<±0.25	12,764
Thioridazine	10	<5	<5	<±0.25	<±0.25	13,689

Topiramate	500	MT (1/9)	MT (1/9)	MT (1/9)	MT (1/9)	911
Trihexyphenidyl	5	<5	<5	<±0.25	<±0.25	23,136
Venlafaxine	50	<5	<5	<±0.25	<±0.25	162,614
Zaleplon	10	<5	<5	<±0.25	<±0.25	13,177
Ziprasidone	10	<5	<5	<±0.25	<±0.25	15,250

Appendix C

Verification Subset Sample B - Blood

Component Name	Conc (ng/mL)	Within Run ppm Error	Between Run ppm Error	Within Run RT Error	Within Run RT Error	Average Response
2C-B	10	MT	MT	MT	MT	MT
2C-E	10	MT	MT	MT	MT	MT
5-MeO-DALT	10	<5	<5	<±0.25	<±0.25	24,062
7-Amino Clonazepam	20	<5	<5	<±0.25	<±0.25	9,818
7-Hydroxymitragynine	10	<5	<5	<±0.25	<±0.25	6,465
9-Hydroxyrisperidone	5	<5	<5	<±0.25	<±0.25	15,866
Acetyl Fentanyl	0.5	>5 (1/9)	>5 (1/9)	<±0.25	<±0.25	2,374
Alfentanil	10	<5	<5	<±0.25	<±0.25	58,580
Alprazolam	20	<5	<5	<±0.25	<±0.25	12,498
Aripiprazole	50	<5	<5	<±0.25	<±0.25	43,428
Benzoylecgonine	100	<5	<5	<±0.25	<±0.25	564
Benztropine	100	<5	<5	<±0.25	<±0.25	448,685
Brompheniramine	10	<5	<5	<±0.25	<±0.25	11,982
Buprenorphine	1	<5	<5	<±0.25	<±0.25	4,533
Butorphanol	2	<5	<5	<±0.25	<±0.25	4,995
BZP	10	<5	<5	<±0.25	<±0.25	4,648
Carbamazepine	200	<5	<5	<±0.25	<±0.25	247,037
Carbamazepine-10, 11 Epoxide	1,000	<5	<5	<±0.25	<±0.25	225,789
Carisoprodol	200	<5	<5	<±0.25	<±0.25	8,931
Chlordiazepoxide	100	<5	<5	<±0.25	<±0.25	70,747

Chlorpheniramine	10	<5	<5	<±0.25	<±0.25	16,146
Clobazam	50	<5	<5	<±0.25	<±0.25	38,611
Cocaine	20	<5	<5	<±0.25	<±0.25	76,040
Cotinine	1,000	<5	<5	<±0.25	<±0.25	88,223
Cyclobenzaprine	20	<5	<5	<±0.25	<±0.25	83,528
Desalkylflurazepam	25	<5	<5	<±0.25	<±0.25	5,482
Desmethylsertraline	20	MT	MT	MT	MT	MT
Diphenhydramine	50	<5	<5	<±0.25	<±0.25	21,793
Donepezil	10	<5	<5	<±0.25	<±0.25	57,869
Doxepin	25	<5	<5	<±0.25	<±0.25	117,774
Emetine	5	<5	<5	<±0.25	<±0.25	12,217
Ephedrine / Pseudoephedrine	250	<5	<5	<±0.25	<±0.25	7,297
Glipizide	100	MT	MT	MT	MT	MT
Guaifenesin	5,000	<5	<5	<±0.25	<±0.25	1,877
Haloperidol	10	<5	<5	<±0.25	<±0.25	37,431
Hydromorphone	10	<5	<5	<±0.25	<±0.25	2,342
Hydroxytriazolam	10	<5	<5	<±0.25	<±0.25	1,988
Iloperidone	10	<5	<5	<±0.25	<±0.25	54,991
Itraconazole	1,000	<5	<5	<±0.25	<±0.25	148,738
Ketamine	10	<5	<5	<±0.25	<±0.25	8,277
Ketoprofen	500,000	<5	<5	<±0.25	<±0.25	87,994
Maprotiline	100	<5	<5	<±0.25	<±0.25	309,951
MDPV	10	<5	<5	<±0.25	<±0.25	25,093
Mepivacaine	1,000	<5	<5	<±0.25	<±0.25	638,690

Metaxalone	250	<5	<5	<±0.25	<±0.25	18,041
Methedrone	10	<5	<5	<±0.25	<±0.25	7,154
Methocarbamol	5,000	<5	<5	<±0.25	<±0.25	7,383
Methylone	10	<5	<5	<±0.25	<±0.25	7,005
Midazolam	10	<5	<5	<±0.25	<±0.25	29,946
Monoethylglycinexylidide (MEGX)	1,000	<5	<5	<±0.25	<±0.25	186,240
Nalbuphine	10	<5	<5	<±0.25	<±0.25	23,917
Norclozapine	25	<5	<5	<±0.25	<±0.25	66,334
Normeperidine	100	<5	<5	>±0.25	>±0.25	167,116
Nortriptyline	50	<5	<5	<±0.25	<±0.25	174,708
Orphenadrine	50	<5	<5	<±0.25	<±0.25	21,209
Oxycodone	10	<5	<5	<±0.25	<±0.25	7,862
Paroxetine	20	<5	<5	<±0.25	<±0.25	58,259
Pentazocine	100	<5	<5	<±0.25	<±0.25	326,946
Perphenazine	5	<5	<5	<±0.25	<±0.25	7,588
Phenazepam	10	<5	<5	<±0.25	<±0.25	1,239
Phendimetrazine	10	<5	<5	<±0.25	<±0.25	7,991
Phensuximide	2,000	<5	<5	<±0.25	<±0.25	4,284
Phentermine	50	MT	MT	MT	MT	MT
Phenyltoloxamine	50	<5	<5	<±0.25	<±0.25	166,256
Primidone	2,500	<5	<5	<±0.25	<±0.25	13,023
Procainamide	5,000	<5	<5	<±0.25	<±0.25	512,076
Propoxyphene	250	<5	<5	<±0.25	<±0.25	185,575
Psilocin	10	MT(5/9)	MT(5/9)	MT(5/9)	MT(5/9)	331

Strychnine	100	<5	<5	<±0.25	<±0.25	329,998
Ticlopidine	100	<5	<5	<±0.25	<±0.25	167,260
Tranylcypromine	50	MT	MT	MT	MT	MT
Triazolam	10	<5	<5	<±0.25	<±0.25	12,301
Trifluoperazine	5	<5	<5	>±0.25	>±0.25	9,653
Trimipramine	50	<5	<5	<±0.25	<±0.25	163,466
Warfarin	250	<5	<5	<±0.25	<±0.25	3,358
Xylazine	5	<5	<5	<±0.25	<±0.25	6,880
Zonisamide	250	<5	<5	<±0.25	<±0.25	868

Verification Subset Sample C - Blood

Component Name (VSS C Blood)	Conc (ng/mL)	Within Run ppm Error	Between Run ppm Error	Within Run RT Error	Between Run RT Error	Average Response
10-Hydroxycarbazepine	10	<5	<5	<±0.25	<±0.25	19,573
1-Hydroxymidazolam	10	<5	<5	<±0.25	<±0.25	9,090
Alpha-PVP	10	<5	<5	<±0.25	<±0.25	18,583
Amphetamine	20	MT	MT	MT	MT	MT
Benzocaine	10	<5	<5	>±0.25	>±0.25	6,567
Bupivacaine	5	>5 (1/9)	>5 (1/9)	<±0.25	<±0.25	599,750
Bupropion	0.5	<5	<5	<±0.25	<±0.25	24,185
Buspirone	10	<5	<5	<±0.25	<±0.25	159,166
Caffeine	20	<5	<5	<±0.25	<±0.25	44,165
Citalopram / Escitalopram	50	<5	<5	<±0.25	<±0.25	448,511
Clonazepam	100	<5	<5	<±0.25	<±0.25	3,662
Clonidine	100	<5	<5	<±0.25	<±0.25	5,568
Codeine	10	<5	<5	<±0.25	<±0.25	28,865
Desmethylclomipramine	1	<5	<5	<±0.25	<±0.25	136,793
Desmethyldoxepin	2	<5	<5	<±0.25	<±0.25	83,722
Dextro / Levo Methorphan	10	<5	<5	<±0.25	<±0.25	219,578
Diacetylmorphine	200	<5	<5	<±0.25	<±0.25	7,919
Diazepam	1,000	<5	<5	<±0.25	<±0.25	35,285
Dicyclomine	200	<5	<5	<±0.25	<±0.25	432,537
Didesmethylsibutramine	100	MT (5/9)	MT (5/9)	MT (5/9)	MT (5/9)	454

Duloxetine	10	<5	<5	<±0.25	<±0.25	14,863
Fentanyl	50	<5	<5	<±0.25	<±0.25	4,850
Flecainide	20	<5	<5	<±0.25	<±0.25	1,059,555
Flurazepam	1,000	<5	<5	<±0.25	<±0.25	48,775
Glimepiride	20	MT (6/9)	MT (6/9)	MT (6/9)	MT (6/9)	458
Glutethimide	25	<5	<5	<±0.25	<±0.25	6,091
Hydrocodone	50	<5	<5	<±0.25	<±0.25	17,212
Hydroxyzine	10	<5	<5	<±0.25	<±0.25	107,438
Ketoconazole	25	<5	<5	>±0.25 (3/9)	>±0.25 (3/9)	1,132,689
Lamotrigine	5	<5	<5	<±0.25	<±0.25	126,954
Levamisole	250	<5	<5	<±0.25	<±0.25	219,482
Lorazepam	100	<5	<5	<±0.25	<±0.25	711
LSD	5,000	<5	<5	<±0.25	<±0.25	9,806
mCPP	10	<5	<5	<±0.25	<±0.25	24,837
MDMA	10	<5	<5	<±0.25	<±0.25	4,453
Memantine	10	<5	<5	<±0.25	<±0.25	1,827
Meperidine	10	<5	<5	<±0.25	<±0.25	298,874
Mephedrone	1,000	<5	<5	<±0.25	<±0.25	5,810
Methadone	10	<5	<5	<±0.25	<±0.25	225,942
Methamphetamine	10	<5	<5	<±0.25	<±0.25	1,457
Mexiletine	100	<5	<5	<±0.25	<±0.25	42,069
Mirtazapine	10	<5	<5	<±0.25	<±0.25	74,013
Naloxone	1,000	<5	<5	<±0.25	<±0.25	866
Nifedipine	250	<5	<5	<±0.25	<±0.25	3,699

Norflunitrazepam	10	<5	<5	<±0.25	<±0.25	1,974
Norpseudoephedrine / Phenylpropanolamine	5,000	<5	<5	<±0.25	<±0.25	455
O-Desmethylvenlafaxine	10	<5	<5	<±0.25	<±0.25	107,549
Phenmetrazine	10	<5	<5	<±0.25	<±0.25	10,915
Phenytoin	1,000	<5	<5	<±0.25	<±0.25	1,235
Prochlorperazine	10	<5	<5	<±0.25	<±0.25	21,446
Promazine	25	<5	<5	<±0.25	<±0.25	155,980
Promethazine	100	<5	<5	<±0.25	<±0.25	13,138
Protriptyline	50	<5	<5	<±0.25	<±0.25	69,682
Pyrilamine	50	MT	MT	MT	MT	MT
Quinidine	10	<5	<5	<±0.25	<±0.25	1,176,125
Ramelteon	20	<5	<5	<±0.25	<±0.25	2,729
Salvinorin B	100	MT	MT	MT	MT	MT
Sertraline	5	<5	<5	<±0.25	<±0.25	7,010
Sibutramine	10	<5	<5	<±0.25	<±0.25	6,393
Sufentanil	10	<5	<5	<±0.25	<±0.25	6,765
Tadalafil	2,000	<5	<5	<±0.25	<±0.25	2,290
Tetrahydrozoline	50	<5	<5	<±0.25	<±0.25	1,236
TFMPP	50	<5	<5	>±0.25 (5/9)	>±0.25 (5/9)	15,688
Theophylline	2,500	<5	<5	<±0.25	<±0.25	1,426
Tramadol	10	<5	<5	<±0.25	<±0.25	45,688
Trazodone	100	<5	<5	<±0.25	<±0.25	382,461
Triprolidine	100	<5	<5	<±0.25	<±0.25	25,231

Vardenafil	50	<5	<5	<±0.25	<±0.25	77,215
Verapamil	10	<5	<5	<±0.25	<±0.25	97,737
Voriconazole	5	<5	<5	<±0.25	<±0.25	492,260
Yohimbine	50	<5	<5	<±0.25	<±0.25	13,177
Zolpidem	250	<5	<5	<±0.25	<±0.25	47,000

Verification Subset Sample D - Blood

Component Name	Conc (ng/mL)	Within Run ppm Error	Between Run ppm Error	Within Run RT Error	Between Run RT Error	Average Response
25I-NBOMe	1	<5	<5	<±0.25	<±0.25	3,927
25C-NBOMe	1	<5	<5	<±0.25	<±0.25	3,490
25B-NBOMe	1	<5	<5	<±0.25	<±0.25	2,546
2C-B	10	<5	<5	<±0.25	<±0.25	383
2C-B-FLY	10	MT (4/9)	MT (4/9)	MT (4/9)	MT (4/9)	368
2C-C	10	MT	MT	MT	MT	MT
2C-E	10	MT (3/9)	MT (3/9)	MT (3/9)	MT (3/9)	378
2C-H	10	MT	MT	MT	MT	MT
2C-I	10	<5	<5	<±0.25	<±0.25	1,016
2C-N	10	<5	<5	<±0.25	<±0.25	674
2C-P	10	<5	<5	<±0.25	<±0.25	439
2C-T-2	10	MT	MT	MT	MT	MT
2C-T-7	10	MT (7/9)	MT (7/9)	MT (7/9)	MT (7/9)	352
3,4-DMMC	10	<5	<5	<±0.25	<±0.25	1,842
5-MeO-DALT	10	<5	<5	<±0.25	<±0.25	25,374
5-MeO-DiPT/Ropivicaine	10	<5	<5	<±0.25	<±0.25	28,765
5-MeO-DMT	10	<5	<5	<±0.25	<±0.25	6,682
7-Hydroxymitragynine	10	<5	<5	<±0.25	<±0.25	10,958
alpha-PVP	2	<5	<5	<±0.25	<±0.25	3,279
Amphetamine	10	MT	MT	MT	MT	MT
AMT	10	MT	MT	MT	MT	MT
Atropine	10	<5	<5	<±0.25	<±0.25	32,648

BDB	10	>5 (1/9)	>5 (1/9)	<±0.25	<±0.25	1,138
Benzoylecgonine	100	<5	<5	<±0.25	<±0.25	564
Bromo-Dragon FLY	10	<5	<5	>±0.25	>±0.25	3,871
Bufotenine	10	<5	<5	<±0.25	<±0.25	2,578
Butylone	10	<5	<5	<±0.25	<±0.25	10,841
BZP	10	<5	<5	<±0.25	<±0.25	3,928
Cathinone	10	MT	MT	MT	MT	MT
Cocaine	10	<5	<5	<±0.25	<±0.25	33,953
DBZP	10	<5	<5	<±0.25	<±0.25	30,307
Dextro / Levo Methorphan	150	<5	<5	<±0.25	<±0.25	44,7033
Dextrorphan / Levorphanol	100	<5	<5	<±0.25	<±0.25	25,8948
DMA	10	<5	<5	<±0.25	<±0.25	5,297
DMAA	50	MT	MT	MT	MT	MT
DMT/Fenproporex	10	<5	<5	<±0.25	<±0.25	2,331
DOB	10	<5	<5	<±0.25	<±0.25	5,095
DOM	10	<5	<5	<±0.25	<±0.25	4,131
Ethylone	10	<5	<5	<±0.25	<±0.25	7,736
Ketamine	10	<5	<5	<±0.25	<±0.25	15,930
LSD	2	<5	<5	<±0.25	<±0.25	8,899
MBDB	10	<5	<5	<±0.25	<±0.25	9,359
MBZP	10	<5	<5	<±0.25	<±0.25	4,120
mCPP	10	<5	<5	<±0.25	<±0.25	6,820
MDA	10	MT (2/9)	MT (2/9)	MT (2/9)	MT (2/9)	372
MDEA	10	<5	<5	<±0.25	<±0.25	10,034

MDMA	10	<5	<5	<±0.25	<±0.25	4,065
MDPV	10	<5	<5	<±0.25	<±0.25	35,032
Mephedrone	10	<5	<5	<±0.25	<±0.25	1,132
Mescaline	50	MT (7/9)	MT (7/9)	MT (7/9)	MT (7/9)	630
Methamphetamine	10	<5	<5	<±0.25	<±0.25	1,396
Methcathinone	10	MT	MT	MT	MT	MT
Methoxetamine	2	<5	<5	<±0.25	<±0.25	4,189
Methylone	10	<5	<5	<±0.25	<±0.25	3,614
Mitragynine	10	<5	<5	<±0.25	<±0.25	46,021
Norketamine	10	<5	<5	<±0.25	<±0.25	2,508
O-Desmethyltramadol	10	<5	<5	<±0.25	<±0.25	18,583
Pentedrone/4-MEC	2	<5	<5	<±0.25	<±0.25	576
Pentylone	10	<5	<5	<±0.25	<±0.25	18,921
Phenazepam	10	<5	<5	<±0.25	<±0.25	1,268
Phencyclidine	10	<5	<5	<±0.25	<±0.25	4,422
PMA	10	MT	MT	MT	MT	MT
Psilocin	10	<5	<5	<±0.25	<±0.25	545
Pyrovalerone	10	<5	<5	<±0.25	<±0.25	34,708
Salvinorin B	2	MT	MT	MT	MT	MT
Scopolamine	10	<5	<5	<±0.25	<±0.25	26,520
TFMPP	10	<5	<5	>±0.25(6/9)	>±0.25(6/9)	15,159
Tramadol	50	<5	<5	<±0.25	<±0.25	112,898
Trazodone	100	<5	<5	<±0.25	<±0.25	358,629

QTOF Verification-Urine

Verification Subset Sample A - Urine

Component Name	Conc (ng/mL)	Within Run ppm Error	Between Run ppm Error	Within Run RT Error	Between Run RT Error	Average Response
6-Monoacetylmorphine	10	<5	<5	<±0.25	<±0.25	16,984
7-Amino Flunitrazepam	10	<5	<5	<±0.25	<±0.25	14,350
Acetaminophen	20,000	<5	<5	<±0.25	<±0.25	58,024
Alpha-Hydroxyalprazolam	20	<5	<5	<±0.25	<±0.25	1,440
Amitriptyline	50	<5	<5	<±0.25	<±0.25	179,744
Amoxapine	50	<5	<5	<±0.25	<±0.25	63,087
Atomoxetine	100	<5	<5	<±0.25	<±0.25	90,229
Atropine	1,000	<5	<5	<±0.25	<±0.25	764,591
Bromo-Dragon FLY	10	<5	<5	<±0.25	<±0.25	3,865
Cephaeline	5	<5	<5	<±0.25	<±0.25	6,744
Chlorpromazine	20	<5	<5	<±0.25	<±0.25	42,997
Clomipramine	50	<5	<5	<±0.25	<±0.25	124,223
Clozapine	50	<5	<5	<±0.25	<±0.25	147,818
Cocaethylene	20	<5	<5	<±0.25	<±0.25	83,424
Desipramine	50	<5	<5	<±0.25	<±0.25	130,628
Dextrorphan / Levorphanol	100	<5	<5	<±0.25	<±0.25	205,566
Dihydrocodeine / Hydrocodol	10	<5	<5	<±0.25	<±0.25	23,010
Diltiazem	100	<5	<5	<±0.25	<±0.25	447,531
Doxylamine	50	<5	<5	<±0.25	<±0.25	14,462

EDDP	50	<5	<5	<±0.25	<±0.25	56,343
Estazolam	20	<5	<5	<±0.25	<±0.25	8,465
Eszopiclone / Zopiclone	10	<5	<5	>±0.25 (1/9)	>±0.25 (1/9)	990
Etodolac	50,000	<5	<5	<±0.25	<±0.25	4,879
Flunitrazepam	5	<5	<5	<±0.25	<±0.25	4,260
Fluoxetine	50	<5	<5	<±0.25	<±0.25	111,256
Fluphenazine	5	<5	<5	<±0.25	<±0.25	13,402
Fluvoxamine	250	<5	<5	<±0.25	<±0.25	187,627
Hydroxybupropion	100	<5	<5	<±0.25	<±0.25	49,565
Hydroxyethylflurazepam	25	<5	<5	<±0.25	<±0.25	7,717
Imipramine	25	<5	<5	<±0.25	<±0.25	86,810
Indomethacin	5,000	<5	<5	<±0.25	<±0.25	474
Lacosamide	10	<5	<5	<±0.25	<±0.25	1,428
Laudanosine	10	<5	<5	<±0.25	<±0.25	1,802
Levetiracetam	5,000	MT	MT	MT	MT	MT
Lidocaine	1,000	<5	<5	<±0.25	<±0.25	527,725
Loxapine	50	<5	<5	<±0.25	<±0.25	164,837
MDA	10	<5	<5	<±0.25	<±0.25	345
MDEA	10	<5	<5	<±0.25	<±0.25	3,919
Meprobamate	1,000	<5	<5	<±0.25	<±0.25	830
Mescaline	10	MT	MT	MT	MT	MT
Mesoridazine	100	<5	<5	<±0.25	<±0.25	367,976
Methaqualone	5,000	>5 (3/9)	>5 (3/9)	<±0.25	<±0.25	1,195,076
Methcathinone	10	<5	<5	<±0.25	<±0.25	3,320
Methylphenidate	10	<5	<5	<±0.25	<±0.25	21,103

Metoclopramide	10	<5	<5	<±0.25	<±0.25	22,302
Mitragynine	10	<5	<5	<±0.25	<±0.25	45,679
Morphine	10	<5	<5	<±0.25	<±0.25	1,915
Naltrexone	1	<5	<5	<±0.25	<±0.25	1,534
Naproxen	500,000	<5	<5	<±0.25	<±0.25	3,278
Nicotine	100	<5	<5	<±0.25	<±0.25	17,707
Norbuprenorphine	2	<5	<5	<±0.25	<±0.25	2,749
Nordiazepam	25	<5	<5	<±0.25	<±0.25	5,551
Norfentanyl	1	<5	<5	<±0.25	<±0.25	505
Norfluoxetine	100	<5	<5	<±0.25	<±0.25	3,424
Norketamine	20	<5	<5	<±0.25	<±0.25	875
Norpropoxyphene	250	<5	<5	<±0.25	<±0.25	14,689
O-Desmethyltramadol	25	<5	<5	<±0.25	<±0.25	43,480
Oxazepam	50	<5	<5	<±0.25	<±0.25	3,131
Oxymorphone	10	<5	<5	<±0.25	<±0.25	2,586
Papaverine	500	>5 (1/9)	>5 (1/9)	<±0.25	<±0.25	1,164,256
Phencyclidine	5	<5	<5	<±0.25	<±0.25	1,998
Pheniramine	10	<5	<5	<±0.25	<±0.25	10,093
PMA	10	MT	MT	MT	MT	MT
Quetiapine	100	<5	<5	<±0.25	<±0.25	425,049
Quinine	2,000	>5 (1/9)	>5 (1/9)	<±0.25	<±0.25	1,165,779
Risperidone	5	<5	<5	<±0.25	<±0.25	20,851
Sildenafil	50	<5	<5	<±0.25	<±0.25	38,090
Tapentadol	10	<5	<5	<±0.25	<±0.25	16,449
Temazepam	50	<5	<5	<±0.25	<±0.25	10,454

Thioridazine	10	>5 (1/9)	>5 (1/9)	<±0.25	<±0.25	6,726
Topiramate	500	<5	<5	<±0.25	<±0.25	1,164
Trihexyphenidyl	5	<5	<5	<±0.25	<±0.25	20,819
Venlafaxine	50	<5	<5	<±0.25	<±0.25	140,783
Zaleplon	10	<5	<5	<±0.25	<±0.25	11,352
Ziprasidone	10	<5	<5	<±0.25	<±0.25	13,459

Verification Subset Sample B - Urine

Component Name	Conc (ng/mL)	Within Run PPM Error	Between Run PPM Error	Within Run RT Error	Within Run RT Error	Average Response
2C-B	10	<5	<5	<±0.25	<±0.25	2,615
2C-E	10	<5	<5	<±0.25	<±0.25	1,726
5-MeO-DALT	10	<5	<5	<±0.25	<±0.25	13,459
7-Amino Clonazepam	20	<5	<5	<±0.25	<±0.25	9,620
7-Hydroxymitragynine	10	MT	MT	MT	MT	MT
9-Hydroxyrisperidone	5	<5	<5	<±0.25	<±0.25	13,121
Acetyl Fentanyl	0.5	<5	<5	<±0.25	<±0.25	1,986
Alfentanil	10	<5	<5	<±0.25	<±0.25	48,281
Alprazolam	20	<5	<5	<±0.25	<±0.25	10,037
Aripiprazole	50	<5	<5	<±0.25	<±0.25	29,297
Benzoylecgonine	100	<5	<5	<±0.25	<±0.25	423
Benztropine	100	<5	<5	<±0.25	<±0.25	387,906
Brompheniramine	10	<5	<5	<±0.25	<±0.25	11,158
Buprenorphine	1	<5	<5	<±0.25	<±0.25	3,331
Butorphanol	2	<5	<5	<±0.25	<±0.25	4,351
BZP	10	<5	<5	<±0.25	<±0.25	3,853
Carbamazepine	200	<5	<5	<±0.25	<±0.25	217,221
Carbamazepine-10, 11 Epoxide	1,000	<5	<5	<±0.25	<±0.25	132,472
Carisoprodol	200	<5	<5	<±0.25	<±0.25	7,674
Chlordiazepoxide	100	<5	<5	<±0.25	<±0.25	14,213

Chlorpheniramine	10	<5	<5	<±0.25	<±0.25	14,213
Clobazam	50	<5	<5	<±0.25	<±0.25	32,392
Cocaine	20	<5	<5	<±0.25	<±0.25	67,468
Cotinine	1,000	<5	<5	<±0.25	<±0.25	73,672
Cyclobenzaprine	20	<5	<5	<±0.25	<±0.25	71,134
Desalkylflurazepam	25	<5	<5	<±0.25	<±0.25	3,988
Desmethylsertraline	20	MT	MT	MT	MT	MT
Diphenhydramine	50	<5	<5	<±0.25	<±0.25	18,383
Donepezil	10	<5	<5	<±0.25	<±0.25	48,574
Doxepin	25	<5	<5	<±0.25	<±0.25	99,023
Emetine	5	<5	<5	<±0.25	<±0.25	9,464
Ephedrine / Pseudoephedrine	250	<5	<5	<±0.25	<±0.25	6,362
Glipizide	100	MT	MT	MT	MT	MT
Guaifenesin	5,000	<5	<5	<±0.25	<±0.25	1,759
Haloperidol	10	<5	<5	<±0.25	<±0.25	30,968
Hydromorphone	10	<5	<5	<±0.25	<±0.25	2,250
Hydroxytriazolam	10	<5	<5	<±0.25	<±0.25	1,692
Iloperidone	10	<5	<5	<±0.25	<±0.25	43,293
Itraconazole	1,000	<5	<5	<±0.25	<±0.25	24,070
Ketamine	10	<5	<5	<±0.25	<±0.25	7,285
Ketoprofen	500,000	<5	<5	<±0.25	<±0.25	97,118
Maprotiline	100	<5	<5	<±0.25	<±0.25	265,698
MDPV	10	<5	<5	<±0.25	<±0.25	20,971
Mepivacaine	1,000	<5	<5	<±0.25	<±0.25	588,896

Metaxalone	250	<5	<5	<±0.25	<±0.25	16,079
Methedrone	10	<5	<5	<±0.25	<±0.25	6,083
Methocarbamol	5,000	<5	<5	<±0.25	<±0.25	5,933
Methylone	10	<5	<5	<±0.25	<±0.25	5,933
Midazolam	10	<5	<5	<±0.25	<±0.25	25,412
Monoethylglycinexylidide (MEGX)	1,000	<5	<5	<±0.25	<±0.25	167,051
Nalbuphine	10	<5	<5	<±0.25	<±0.25	21,197
Norclozapine	25	<5	<5	<±0.25	<±0.25	53,075
Normeperidine	100	<5	<5	<±0.25	<±0.25	138,066
Nortriptyline	50	<5	<5	<±0.25	<±0.25	18,619
Orphenadrine	50	<5	<5	<±0.25	<±0.25	18,619
Oxycodone	10	<5	<5	<±0.25	<±0.25	6,886
Paroxetine	20	<5	<5	<±0.25	<±0.25	42,495
Pentazocine	100	<5	<5	<±0.25	<±0.25	286,710
Perphenazine	5	<5	<5	<±0.25	<±0.25	6,496
Phenazepam	10	<5	<5	<±0.25	<±0.25	1,079
Phendimetrazine	10	<5	<5	<±0.25	<±0.25	7,008
Phensuximide	2,000	<5	<5	<±0.25	<±0.25	2,923
Phentermine	50	MT	MT	MT	MT	MT
Phenyltoloxamine	50	<5	<5	<±0.25	<±0.25	140,784
Primidone	2,500	<5	<5	<±0.25	<±0.25	11,518
Procainamide	5,000	<5	<5	<±0.25	<±0.25	471,646
Propoxyphene	250	<5	<5	<±0.25	<±0.25	157,289
Psilocin	10	MT	MT	MT	MT	MT

Strychnine	100	<5	<5	<±0.25	<±0.25	286,874
Ticlopidine	100	<5	<5	<±0.25	<±0.25	140,156
Tranylcypromine	50	MT	MT	MT	MT	MT
Triazolam	10	<5	<5	<±0.25	<±0.25	9,477
Trifluoperazine	5	<5	<5	>±0.25	>±0.25	8,209
Trimipramine	50	<5	<5	<±0.25	<±0.25	141,428
Warfarin	250	<5	<5	<±0.25	<±0.25	4,333
Xylazine	5	<5	<5	<±0.25	<±0.25	6,618
Zonisamide	250	<5	<5	<±0.25	<±0.25	957

Appendix C

Verification Subset Sample C - Urine

Component Name (VSS C Urine)	Conc. (ng/mL)	Within Run ppm Error	Between Run ppm Error	Within Run RT Error	Between run RT Error	Average Response
10-Hydroxycarbazepine	10	<5	<5	<±0.25	<±0.25	17,529
1-Hydroxymidazolam	10	<5	<5	<±0.25	<±0.25	6,971
Alpha-PVP	10	<5	<5	>±0.25 (6/9)	>±0.25 (6/9)	15,481
Amphetamine	20	MT	MT	MT	MT	MT
Benzocaine	10	<5	<5	<±0.25	<±0.25	4,814
Bupivacaine	5	<5	<5	<±0.25	<±0.25	544,909
Bupropion	0.5	<5	<5	<±0.25	<±0.25	19,653
Buspirone	10	<5	<5	<±0.25	<±0.25	131,924
Caffeine	20	<5	<5	<±0.25	<±0.25	36,070
Citalopram / Escitalopram	50	<5	<5	<±0.25	<±0.25	392,160
Clonazepam	100	<5	<5	<±0.25	<±0.25	3,097
Clonidine	100	<5	<5	<±0.25	<±0.25	4,802
Codeine	10	<5	<5	<±0.25	<±0.25	24,598
Desmethylclomipramine	1	<5	<5	<±0.25	<±0.25	113,983
Desmethyldoxepin	2	<5	<5	<±0.25	<±0.25	69,869
Dextro / Levo Methorphan	10	<5	<5	<±0.25	<±0.25	179,681
Diacetylmorphine	200	<5	<5	<±0.25	<±0.25	6,612
Diazepam	1,000	<5	<5	<±0.25	<±0.25	29,139
Dicyclomine	200	<5	<5	<±0.25	<±0.25	394,382
Didesmethylsibutramine	100	<5	<5	<±0.25	<±0.25	342

Duloxetine	10	<5	<5	<±0.25	<±0.25	12,197
Fentanyl	50	<5	<5	<±0.25	<±0.25	4,126
Flecainide	20	<5	<5	<±0.25	<±0.25	964,749
Flurazepam	1,000	<5	<5	<±0.25	<±0.25	39,469
Glimepiride	20	<5	<5	<±0.25	<±0.25	536
Glutethimide	25	<5	<5	<±0.25	<±0.25	5,144
Hydrocodone	50	<5	<5	<±0.25	<±0.25	14,319
Hydroxyzine	10	<5	<5	<±0.25	<±0.25	88,126
Ketoconazole	25	<5	<5	<±0.25	<±0.25	937,279
Lamotrigine	5	<5	<5	<±0.25	<±0.25	158,929
Levamisole	250	>5 (1/9)	>5 (1/9)	<±0.25	<±0.25	197,354
Lorazepam	100	<5	<5	<±0.25	<±0.25	593
LSD	5,000	<5	<5	<±0.25	<±0.25	7,759
mCPP	10	<5	<5	<±0.25	<±0.25	26,274
MDMA	10	<5	<5	<±0.25	<±0.25	3,783
Memantine	10	<5	<5	<±0.25	<±0.25	1,444
Meperidine	10	<5	<5	<±0.25	<±0.25	257,583
Mephedrone	1,000	<5	<5	<±0.25	<±0.25	4,878
Methadone	10	<5	<5	<±0.25	<±0.25	193,723
Methamphetamine	10	<5	<5	<±0.25	<±0.25	1,297
Mexiletine	100	<5	<5	<±0.25	<±0.25	35,873
Mirtazapine	10	<5	<5	<±0.25	<±0.25	62,279
Naloxone	1,000	<5	<5	<±0.25	<±0.25	716
Nifedipine	250	<5	<5	<±0.25	<±0.25	2,865
Norflunitrazepam	10	<5	<5	<±0.25	<±0.25	1,363

Norpseudoephedrine / Phenylpropanolamine	5,000	<5	<5	<±0.25	<±0.25	373
O-Desmethylvenlafaxine	10	<5	<5	<±0.25	<±0.25	97,971
Phenytoin	1,000	<5	<5	<±0.25	<±0.25	1,332
Prochlorperazine	10	<5	<5	<±0.25	<±0.25	22,645
Promazine	25	<5	<5	<±0.25	<±0.25	145,482
Promethazine	100	<5	<5	<±0.25	<±0.25	10,908
Protriptyline	50	<5	<5	<±0.25	<±0.25	61,354
Pyrilamine	50	<5	<5	<±0.25	<±0.25	100,925
Quinidine	10	<5	<5	<±0.25	<±0.25	1,102,199
Ramelteon	20	<5	<5	<±0.25	<±0.25	2,380
Salvinorin B	100	MT	MT	MT	MT	MT
Sertraline	5	<5	<5	<±0.25	<±0.25	6,363
Sibutramine	10	<5	<5	<±0.25	<±0.25	8,238
Sufentanil	10	<5	<5	<±0.25	<±0.25	5,844
Tadalafil	2,000	<5	<5	<±0.25	<±0.25	1,708
Tetrahydrozoline	50	<5	<5	<±0.25	<±0.25	1,004
TFMPP	50	<5	<5	<±0.25	<±0.25	12,744
Theophylline	2,500	<5	<5	<±0.25	<±0.25	1,173
Tramadol	10	<5	<5	<±0.25	<±0.25	36,145
Trazodone	100	<5	<5	>±0.25 (3/9)	>±0.25 (3/9)	326,591
Triprolidine	100	<5	<5	<±0.25	<±0.25	19,610
Vardenafil	50	<5	<5	<±0.25	<±0.25	64,625
Verapamil	10	<5	<5	<±0.25	<±0.25	81,898

Voriconazole	5	<5	<5	<±0.25	<±0.25	432,305
Yohimbine	50	<5	<5	<±0.25	<±0.25	8,782
Zolpidem	250	<5	<5	<±0.25	<±0.25	39,076

Appendix C

Verification Subset Sample D – Urine

Component Name	Conc (ng/mL)	Within Run ppm Error	Between Run ppm Error	Within Run RT Error	Between Run RT Error	Average Response
25I-NBOMe	1	<5	<5	<±0.25	<±0.25	2,692
25C-NBOMe	1	<5	<5	<±0.25	<±0.25	2,538
25B-NBOMe	1	<5	<5	<±0.25	<±0.25	1,932
2C-B	10	<5	<5	<±0.25	<±0.25	2,349
2C-B-FLY	10	<5	<5	<±0.25	<±0.25	3,647
2C-C	10	<5	<5	<±0.25	<±0.25	1,444
2C-E	10	<5	<5	<±0.25	<±0.25	2,616
2C-H	10	<5	<5	<±0.25	<±0.25	1,249
2C-I	10	<5	<5	<±0.25	<±0.25	6,597
2C-N	10	<5	<5	<±0.25	<±0.25	1,471
2C-P	10	<5	<5	<±0.25	<±0.25	3,355
2C-T-2	10	<5	<5	<±0.25	<±0.25	1,602
2C-T-7	10	<5	<5	>±0.25	>±0.25	2,286
3,4-DMMC	10	<5	<5	<±0.25	<±0.25	1,737
5-MeO-DALT	10	<5	<5	<±0.25	<±0.25	18,656
5-MeO-DiPT/Ropivicaine	10	<5	<5	<±0.25	<±0.25	22,352
5-MeO-DMT	10	<5	<5	<±0.25	<±0.25	5,245
7-Hydroxymitragynine	10	<5	<5	<±0.25	<±0.25	604
alpha-PVP	2	<5	<5	<±0.25	<±0.25	2,780
Amphetamine	10	MT	MT	MT	MT	MT
AMT	10	MT	MT	MT	MT	MT
Atropine	10	<5	<5	<±0.25	<±0.25	764,591

BDB	10	>5 (2/9)	>5 (2/9)	<±0.25	<±0.25	964
Benzoylecgonine	100	<5	<5	<±0.25	<±0.25	423
Bromo-Dragon FLY	10	<5	<5	<±0.25	<±0.25	3,154
Bufotenine	10	<5	<5	<±0.25	<±0.25	1,837
Butylone	10	<5	<5	<±0.25	<±0.25	11,462
BZP	10	<5	<5	<±0.25	<±0.25	3,440
Cathinone	10	MT	MT	MT	MT	MT
Cocaine	10	<5	<5	<±0.25	<±0.25	30,261
DBZP	10	<5	<5	<±0.25	<±0.25	25,285
DET		<5	<5	<±0.25	<±0.25	7,184
Dextro / Levo Methorphan	150	<5	<5	<±0.25	<±0.25	407,922
Dextrorphan / Levorphanol	100	<5	<5	<±0.25	<±0.25	204,336
DMAA	50	MT	MT	MT	MT	MT
DMT/Fenproporex	10	<5	<5	<±0.25	<±0.25	2,013
DOB	10	<5	<5	<±0.25	<±0.25	4,368
DOM	10	<5	<5	<±0.25	<±0.25	3,439
Ethylone	10	<5	<5	<±0.25	<±0.25	12,463
Ketamine	10	<5	<5	<±0.25	<±0.25	13,675
LSD	2	<5	<5	<±0.25	<±0.25	6,887
MBDB	10	<5	<5	<±0.25	<±0.25	8,056
MBZP	10	<5	<5	<±0.25	<±0.25	3,532
mCPP	10	<5	<5	<±0.25	<±0.25	5,502
MDA	10	<5	<5	<±0.25	<±0.25	345
MDEA	10	<5	<5	<±0.25	<±0.25	8,650

MDMA	10	<5	<5	<±0.25	<±0.25	3,764
MDPV	10	<5	<5	<±0.25	<±0.25	30,081
Mephedrone	10	<5	<5	<±0.25	<±0.25	3,764
Mescaline	50	<5	<5	<±0.25	<±0.25	1,369
Methamphetamine	10	<5	<5	<±0.25	<±0.25	1,302
Methcathinone	10	MT	MT	MT	MT	MT
Methoxetamine	2	<5	<5	<±0.25	<±0.25	3,408
Methylone	10	<5	<5	<±0.25	<±0.25	3,285
Mitragynine	10	<5	<5	<±0.25	<±0.25	34,656
Naphyrone		<5	<5	<±0.25	<±0.25	32,618
Norketamine	10	<5	<5	<±0.25	<±0.25	2,192
O-Desmethyltramadol	10	<5	<5	<±0.25	<±0.25	16,433
Pentedrone/4-MEC	2	<5	<5	<±0.25	<±0.25	478
Pentylone	10	<5	<5	<±0.25	<±0.25	16,252
Phenazepam	10	<5	<5	<±0.25	<±0.25	1,100
Phencyclidine	10	<5	<5	<±0.25	<±0.25	27,831
PMA	10	MT	MT	MT	MT	MT
Psilocin	10	MT	MT	MT	MT	MT
Pyrovalerone	10	<5	<5	<±0.25	<±0.25	27,831
Salvinorin B	2	MT	MT	MT	MT	MT
Scopolamine	10	<5	<5	<±0.25	<±0.25	23,977
TFMPP	10	<5	<5	<±0.25	<±0.25	12,044
Tramadol	50	<5	<5	<±0.25	<±0.25	100,451
Trazodone	100	<5	<5	<±0.25	<±0.25	319,873

Appendix C

QTOF Validation: Oral Fluid

Criteria Evaluated:

1. Precision
    a. Within-Run Precision
    b. Between-Run Precision
2. Processed Sample Stability
3. Matrix Stability
4. Blinds

Components and concentrations:

Compound	Concentration in Saliva(ng/mL)		
	Low	Mid	High
2C-B	25	250	2500
4-Fluoroamphetamine (4-FA)	75	750	7500
alpha-PVP	2	20	200
Benzoylecgonine	1,000	10,000	100,000
Cocaethylene	10	100	1000
Cocaine	5	50	500
Dimethylone	10	100	1000
DOM	25	250	2500
Ketamine	5	50	500
MDA	25	250	2500
MDMA	25	250	2500
Methamphetamine	50	500	5000
Methylone	25	250	2500
Norketamine	25	250	2500
O-Desmethyltramadol	5	50	500
Tramadol	25	250	2500

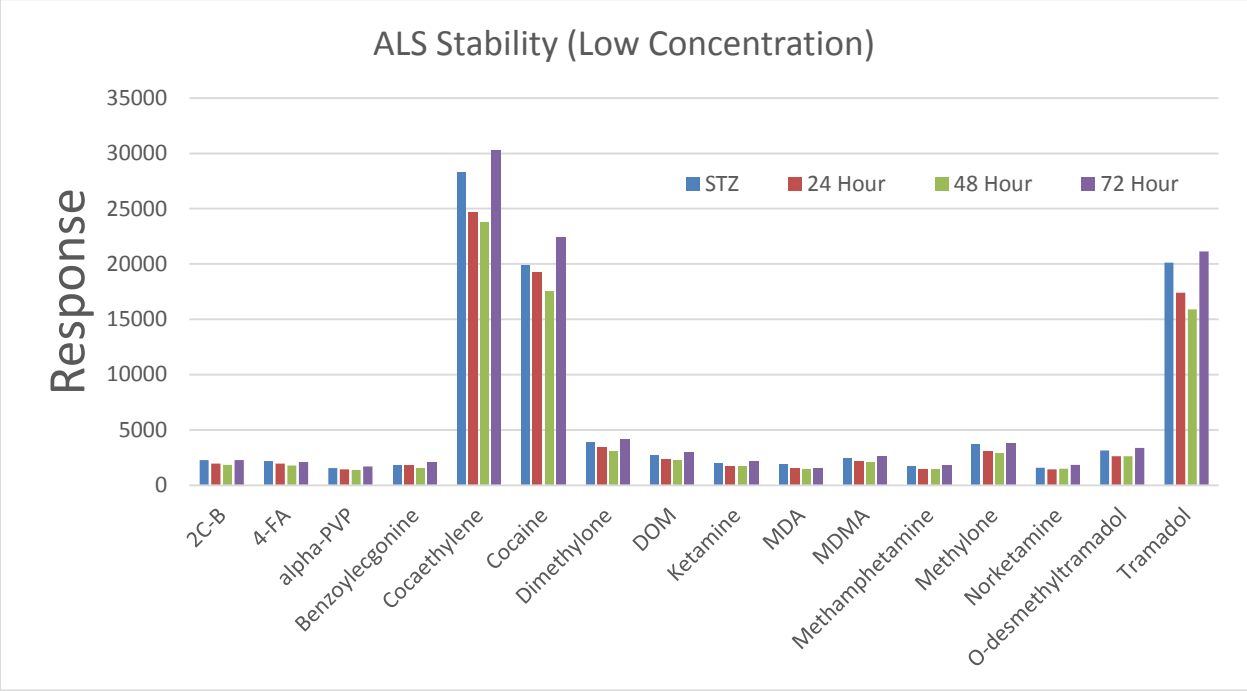
Appendix C

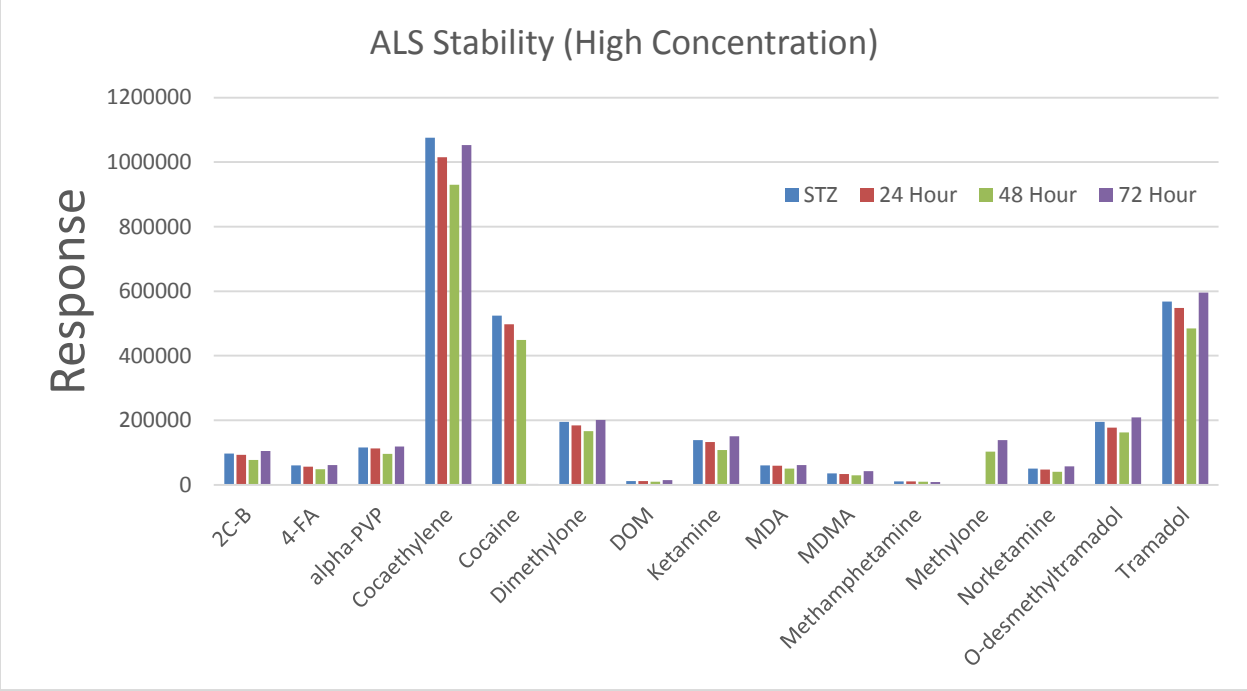
Oral fluid between run precision results over 3 days in triplicate (n=9) by compound

Compound and Level		Fragments		Mass Error	RT Error	Response
		Expected	Average	Average	Average	Average
2C-B	Low	3	2	0.03	-0.22	1971
	Mid	3	2	0.88	-0.22	16895
	High	3	2	1.55	-0.23	93258
4-FA (Fragment as Parent)	Low	3	3	1.24	-0.05	1876
	Mid	3	3	2.37	-0.05	13679
	High	3	2	3.24	-0.07	58579
alpha-PVP	Low	4	2	-1.52	0.04	1439
	Mid	4	3	0.52	0.04	13453
	High	4	4	1.73	0.04	116229
Benzoylegonine	Low	3	1	-0.83	-0.09	1532
	Mid	3	3	0.27	-0.09	12427
Cocaethylene	Low	4	4	1.27	-0.04	24547
	Mid	4	4	2.04	-0.04	224387
	High	4	4	1.70	-0.05	1028272
Cocaine	Low	4	4	0.24	-0.07	9188
	Mid	4	4	0.97	-0.07	74368
	High	4	4	1.65	-0.08	511383
Dimethylone (bkMDDMA)	Low	3	3	0.43	0.00	3524
	Mid	3	3	1.14	0.00	32478
	High	3	3	2.56	0.00	187299
DOM	Low	2	2	-0.32	-0.06	2362
	Mid	2	2	0.94	-0.06	12790
	High	2	0	0.87	-0.03	11893
Ketamine	Low	4	4	-0.37	-0.02	1813
	Mid	4	4	0.68	-0.02	16810
	High	4	4	2.31	-0.02	131576
MDA (Fragment as Parent)	Low	4	4	0.25	-0.04	1565
	Mid	4	4	1.30	-0.04	11673
	High	4	4	2.27	-0.05	60770
MDMA	Low	3	1	0.21	-0.04	2180
	Mid	3	2	0.86	-0.04	13164
	High	3	1	1.41	-0.05	34410
Methamphetamine	Low	2	2	-0.17	0.00	1494
	Mid	2	2	0.81	0.00	7793
	High	2	1	1.22	0.00	11260
Methylone (bkMDMA)	Low	3	2	0.18	-0.14	3254
	Mid	3	3	1.70	-0.14	27807
	High	3	1	0.11	0.04	25245
Norketamine	Low	3	3	-0.38	-0.08	1485
	Mid	3	3	1.21	-0.08	12104
	High	3	3	1.77	-0.08	49019
O-desmethyl tramadol	Low	2	1	-0.10	-0.03	2711
	Mid	2	2	0.58	-0.03	25092
	High	2	2	2.01	-0.04	184833
Tramadol	Low	2	1	0.83	0.07	16979
	Mid	2	2	1.67	0.07	149448
	High	2	2	1.96	0.06	554680

Appendix C

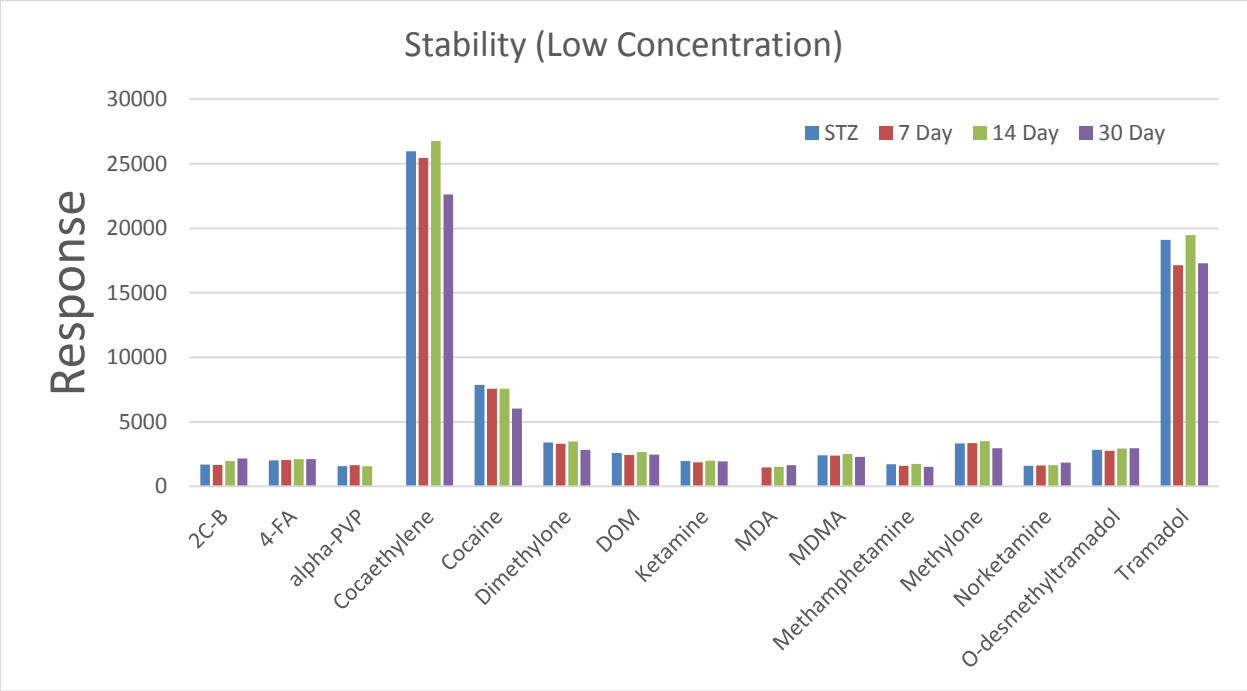
Processed sample stability results

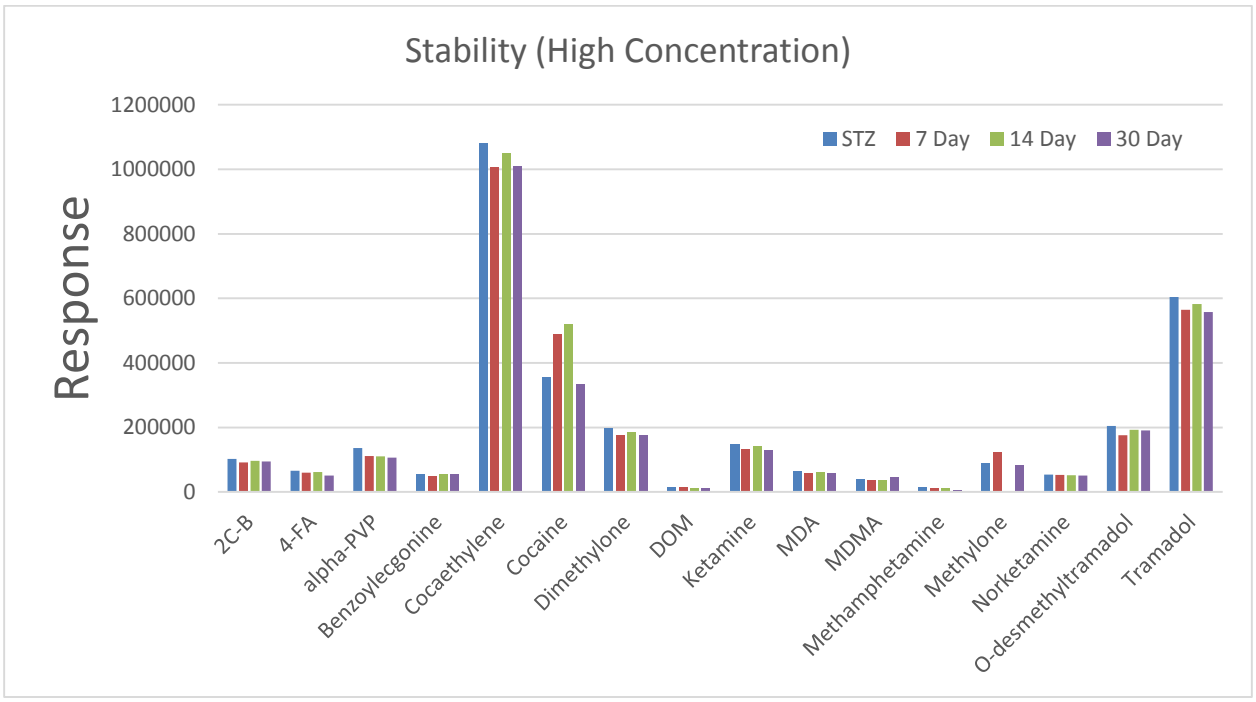




Appendix C

Sample stability for oral fluid samples





Appendix C

Blind oral fluid mock sample validation testing

Blind	Spiked Analytes		Mass Error	RT Error	Fragments		Identified	Other Information
	Compound	Concentration			Exp	Found		
1	Diazepam	20 ng/mL	-0.97	-0.08	3	3	Yes	
	Nordiazepam	40 ng/mL	-0.72	-0.18	4	4	Yes	
2	True Blank	NA	NA	NA	NA	NA	Yes	
3	alpha-PVP	10 ng/mL	0.34	0.03	4	3	Yes	
	Dimethylone	30 ng/mL	1.38	0.00	3	3	Yes	
	Methylone	200 ng/mL	2.10	-0.15	3	3	Yes	
4	Tramadol	10 ng/mL	0.08	0.07	2	2	Yes	Below cutoff
5	Clonazepam	50 ng/mL	-0.15	-0.12	3	3	Yes	
	Amphetamine	250 ng/mL	-	-	-	-	No	
	Dimethylone	30 ng/mL	1.30	0.00	3	3	Yes	
6	alpha-PVP	1 ng/mL	-	-	-	-	No	Below cutoff
	Methylone	10 ng/mL	-	-	-	-	No	Below cutoff
7	True Blank	NA	NA	NA	NA	NA	Yes	
8	Methamphetamine	500 ng/mL	1.26	0.00	2	2	Yes	MDA (Fragment as parent) also identified
	MDMA	150 ng/mL	0.65	-0.05	3	2	Yes	
9	Cocaine	2 ng/mL	-0.15	-0.07	4	4	Yes	Below cutoff
	Benzoylecgonine	50 ng/mL	-	-	-	-	No	Below cutoff
10	Cotinine	1000 ng/mL	2.88	-0.07	4	4	Yes	
	Nicotine	400 ng/mL	2.34	-0.16	4	4	Yes	
11	MDMA	40 ng/mL	-0.46	-0.04	3	1	Yes	
	MDA	30 ng/mL	0.07	0.21	4	2	Yes	(Fragment as parent)
12	Fluoxetine	150 ng/mL	1.57	-0.07	2	1	Yes	
13	Nicotine	100 ng/mL	2.07	-0.16	4	4	Yes	
	Acetaminophen	25000 ng/mL	1.56	-0.02	2	2	Yes	
	Cotinine	1000 ng/mL	2.69	-0.07	4	4	Yes	
14	Oxycodone	20 ng/mL	0.06	-0.02	3	3	Yes	
	Methadone	40 ng/mL	1.83	-0.01	4	4	Yes	
15	Oxycodone	30 ng/mL	0.48	-0.02	3	3	Yes	
	Cocaine	15 ng/mL	1.30	-0.07	4	4	Yes	
	Methylone	40 ng/mL	0.40	-0.14	3	3	Yes	
16	Nicotine	250 ng/mL	2.02	-0.16	4	4	Yes	
	Cotinine	1500 ng/mL	3.92	-0.07	4	4	Yes	
17	MDMA	10 ng/mL	-	-	-	-	No	Below cutoff
18	True Blank	NA	NA	NA	NA	NA	Yes	
19	Dimethylone	50 ng/mL	1.24	0.00	3	3	Yes	
	Methylone	100 ng/mL	1.44	-0.14	3	3	Yes	
20	Cocaine	200 ng/mL	1.94	-0.08	4	4	Yes	
	Benzoylecgonine	1500 ng/mL	-0.72	-0.10	3	3	Yes	
21	Dimethylone	5 ng/mL	1.77	0.00	3	3	Yes	Below cutoff
22	Amphetamine	200 ng/mL	-	-	-	-	No	
	Methamphetamine	200 ng/mL	1.56	0.00	2	2	Yes	
23	Acetaminophen	25000 ng/mL	0.72	-0.03	2	2	Yes	
	Methamphetamine	250 ng/mL	2.20	0.00	2	2	Yes	

Appendix D

Summary of Analytical Findings by Subject

Sample ID	Confirmation in Blood - NMS and The Center	Confirmation in Urine - AFMES	Confirmation in Oral Fluid-NMS and The Center
MS001	-	-	THC (30.7 ng/mL)
MS002	-	Carboxy-THC (>200 ng/mL)	-
MS003	-	-	THC (9.9 ng/mL)
MS004	-	-	THC (65 ng/mL)
MS005	ND	Carboxy-THC (7.4 ng/mL)	ND
MS006	MDMA (7.5 ng/mL) Methylone (168.9 ng/mL) 4-FA (71.1 ng/mL) THC-COOH (9.5 ng/mL)	Carboxy-THC (84.4 ng/mL) MDA (410.3 ng/mL) MDMA (4281.1 ng/mL) Benzoylecgonine (<10 ng/mL) Methylone(>50000 ng/mL) Ethylone (33.7 ng/mL) Butylone (<1 ng/mL) 4-FA (46042.9 ng/mL) Alpha-PVP (72.8 ng/mL) Ethanol (18 mg/dL) Acetone (7 mg/dL)	Methylone (1304.0 ng/mL) 4-FA (378.2 ng/mL) THC (37.2 ng/mL) Ethylone Benzoylecgonine Cocaine MDMA
MS007	-	-	ND
MS008	ND	Carboxy-THC (17.0 ng/mL)	ND
MS009	THC (1.2 ng/mL) THC-COOH (26 ng/mL)	Carboxy-THC (>200 ng/mL)	THC (7.3 ng/mL)
MS010	THC-COOH (<5 ng/mL)	Dextromethorphan (100 ng/mL) Carboxy-THC (28.1 ng/mL) Ethylone (32.8 ng/mL) Dimethylone (1.9 ng/mL)	THC (7.2 ng/mL)
MS011	THC-COOH (14 ng/mL)	Carboxy-THC (39.6 ng/mL) Benzoylecgonine (70.3 ng/mL) Ethanol (4 mg/dL)	THC (57.4 ng/mL)
MS012	Amphetamine (6.9 ng/mL) Methamphetamine (570 ng/mL) THC (13 ng/mL) THC-OH (8.4 ng/mL) THC-COOH (72 ng/mL)	Amphetamine (345.5 ng/mL) Methamphetamine (55287.4 ng/mL) Carboxy-THC (195.0 ng/mL) Methylone (4.8 ng/mL)	Amphetamine Methamphetamine THC (105.4 ng/mL)
MS013	Cocaine (40 ng/mL) Benzoylecgonine (74 ng/mL) Ethanol (25 mg/dL)	Benzoylecgonine (15300.5 ng/mL) Cocaine (4056.0 ng/mL) Methylone (<1 ng/mL) 4-FA (<1 ng/mL) Alpha-PVP (11.0 ng/mL) Ethanol (26 mg/dL)	Benzoylecgonine Cocaine
MS014	THC (1.1 ng/mL) THC-COOH (7.3 ng/mL)	Carboxy-THC (>200 ng/mL)	THC (307.1 ng/mL)

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MS015	THC (6.4 ng/mL) THC-COOH (51 ng/mL) Ethanol (33 mg/dL)	Carboxy-THC (191.9 ng/mL) Ethylone (3.7 ng/mL) Ethanol (29 mg/dL)	THC (7.9 ng/mL)
MS016	-	Amphetamine (<50 ng/mL) Cyclobenzaprine (<50 ng/mL) Carboxy-THC (39.7 ng/mL)	ND
MS017	-	Not Tested	-
MS018	-	-	THC (32 ng/mL)
MS019	-	-	THC (13.5 ng/mL)
MS020	-	-	ND
MS021	-	Ethanol (34 mg/dL)	-
MS022	-	Ethanol (27 mg/dL)	-
MS023	Ethanol (150 mg/dL)	Ethanol (157 mg/dL)	ND
MS024	Ethanol (153 mg/dL)	Ethanol (113 mg/dL)	ND
MS025	THC-COOH (<5 ng/mL)	Carboxy-THC (8.8 ng/mL)	ND
MS026	THC-COOH (<5 ng/mL)	Carboxy-THC (72.6 ng/mL)	ND
MS027	-	Ethanol (138 mg/dL)	ND
MS028	Cocaine (55 ng/mL) Benzoylecgonine (56 ng/mL) Ethanol (67 ng/mL)	-	Benzoylecgonine Cocaethylene Cocaine
MS029	ND	-	ND
MS030	-	-	ND
MS031	ND	ND	ND
MS032	-	-	ND
MS033	-	Azacyclonal (25 ng/mL)	ND
MS034	-	ND	ND
MS035	THC-COOH (7.6 ng/mL)	Carboxy-THC (102.5 ng/mL) Fluoxetine (<50 ng/mL) Benzoylecgonine (57.3 ng/mL) Acetone (4 mg/dL)	ND
MS036	ND	Carboxy-THC (14.4 ng/mL) Benzoylecgonine (<10 ng/mL)	ND
MS037	ND	Carboxy-THC (18.6 ng/mL)	ND
MS038	Ethanol (73 mg/dL)	Carboxy-THC (20.8 ng/mL) Ethanol (100 mg/dL)	ND
MS039	ND	Benzoylecgonine (<10 ng/mL)	ND
MS040	Ethanol (161 mg/dL)	Methylone (1.0 ng/mL) Dimethylone (4.1 ng/mL) Ethanol (203 mg/dL)	ND
MS041	THC (1.2 ng/mL) THC-COOH (27 ng/mL)	Carboxy-THC (137.0 ng/mL)	THC (<2 ng/mL)
MS042	THC (1.3 ng/mL) THC-COOH (24 ng/mL)	Carboxy-THC (>200 ng/mL) Acetone (1 mg/dL)	THC (<2 ng/mL)

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MS043	Ethanol (10 mg/dL)	Methamphetamine (<LOQ) Ethanol (9 mg/dL)	ND
MS044	ND	ND	ND
MS045	THC (1.3 ng/mL) THC-COOH (14 ng/mL)	Carboxy-THC (>200 ng/mL) Benzoylecgonine (14.6 ng/mL)	ND
MS046	ND	ND	ND
MS047	-	MDA (1323.6 ng/mL) MDMA (18538.7 ng/mL) Carboxy-THC (>200 ng/mL) Benzoylecgonine (<10 ng/mL) Methylone (16.1 ng/mL) Dimethylone (<1 ng/mL) Acetone (6 mg/dL)	MDMA THC (2.5 ng/mL)
MS048	Benzoylecgonine (<50 ng/mL) THC (5.1 ng/mL) THC-COOH (25 ng/mL)	Carboxy-THC (>200 ng/mL) Benzoylecgonine (11372.6 ng/mL) Cocaine (66.9 ng/mL)	Benzoylecgonine Cocaine THC (9.6 ng/mL)
MS049	THC (11 ng/mL) Ethanol (32 mg/dL)	Carboxy-THC (59.7 ng/mL) Ethanol (7 mg/dL)	THC (6.7 ng/mL)
MS050	-	Carboxy-THC (166.1 ng/mL) Methylone (41.6 ng/mL)	THC (73.9 ng/mL)
MS051	-	-	Methylone (311.3 ng/mL) THC (201.4 ng/mL)
MS052	-	Carboxy-THC (102.7 ng/mL)	THC (6 ng/ml)
MS053	-	Carboxy-THC (>200 ng/mL) Methylone (17835.0 ng/mL) Ethylone (<1 ng/mL)	Methylone (1154.2 ng/mL) THC (3.8 ng/mL)
MS054	ND	Benzoylecgonine (107.5 ng/mL)	Cocaine
MS055	MDMA (57 ng/mL) MDA (7 ng/mL) THC-COOH (10 ng/mL)	Carboxy-THC (>200 ng/mL) MDA (1315.8 ng/mL) MDMA (12602.7 ng/mL) Methylone (<1 ng/mL) PMMA (<50 ng/mL)	MDA MDMA
MS056	-	Tramadol (290 ng/mL) Benzoylecgonine (493.6 ng/mL) Cocaine (111.0 ng/mL) Cocaethylene (45.1 ng/mL) Methylone (463.4 ng/mL) Ethanol (132 mg/dL)	Benzoylecgonine Cocaethylene Cocaine
MS057	-	Amphetamine (1359.9 ng/mL) PPA (103.9 ng/mL) Benzoylecgonine (1053.0 ng/mL) Methylone (110 ng/mL)	Methylone (6177.3 ng/mL) Amphetamine Benzoylecgonine Cocaine

MS058	-	-	Methylone (118.0 ng/mL) Ethylone (1662.9 ng/mL) THC (140.1 ng/mL) Methamphetamine
MS059	-	Tramadol (1570 ng/mL) Benzoylecgonine (6429.1 ng/mL) Cocaine (500.0 ng/mL) Cocaethylene (12.2 ng/mL) Methylone (842.6 ng/mL) Ethanol (21 mg/dL)	Methylone (3200.3 ng/mL) Benzoylecgonine Cocaethylene Cocaine
MS060	-	ND	ND
MS061	ND	Ethanol (6 mg/dL)	Alpha-PVP
MS062	-	-	Ethylone (335.2 ng/mL)
MS063	-	-	Ethylone (107.1 ng/mL)
MS064	Benzoylecgonine (645 ng/mL) THC (1.7 ng/mL) THC-COOH (8.1 ng/mL) Methylone (29.9 ng/mL) Dimethylone (153 ng/mL)	Carboxy-THC (39.2 ng/mL) Benzoylecgonine (>100000 ng/mL) Cocaine (373.3 ng/mL) Methylone (3389.0 ng/mL) Dimethylone (5369.3 ng/mL)	Methylone (652.6 ng/mL) Dimethylone (511.4 ng/mL) THC (25.4 ng/mL) Benzoylecgonine Cocaine
MS065	THC (1.3 ng/mL) THC-COOH (18 ng/mL) Benzoylecgonine (372.0 ng/mL) Methylone (28.0 ng/mL) Dimethylone (43.2 ng/mL)	Carboxy-THC (118.3 ng/mL) Benzoylecgonine (>100000 ng/mL) Cocaine (3971.5 ng/mL) Methylone (6281.7 ng/mL) Dimethylone (4860.5 ng/mL) Acetone (9 mg/dL)	Methylone (284.7 ng/mL) THC (42.3 ng/mL) Dimethylone Benzoylecgonine Cocaine
MS066	-	ND	THC (6.7 ng/mL)
MS067	-	Fluoxetine (<50 ng/mL)	ND
MS068	-	Carboxy-THC (>200 ng/mL)	THC (10.2 ng/mL)
MS069	-	-	ND
MS070	Alprazolam (57 ng/mL) Oxycodone (Total) (66 ng/mL) Oxymorphone (Total) (19 ng/mL) Methadone (<50 ng/mL)	Methadone (1420 ng/mL) EDDP (570 ng/mL) Oxycodone (>3000 ng/mL) Oxymorphone (>3000 ng/mL) Alprazolam (>1000 ng/mL) 1-OH Alprazolam (592.8 ng/mL)	Alprazolam Oxycodone
MS071	Ethanol (123 mg/dL)	Not Tested	-
MS072	-	-	ND
MS073	ND	ND	ND
MS074	THC (3 ng/mL) THC-COOH (27 ng/mL)	Carboxy-THC (144.8 ng/mL)	THC (<2 ng/mL)

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MS075	THC (2.7 ng/mL) THC-COOH (33 ng/mL) Alpha-PVP (80.5 ng/mL)	Carboxy-THC (>200 ng/mL) MDMA (<LOQ) Psilocin (771.6 ng/mL) Alpha-PVP (2046.5 ng/mL)	Alpha-PVP (379.2 ng/mL) THC (5.4 ng/mL) MDMA
MS076	Cocaine (120 ng/mL) Benzoylecgonine (210 ng/mL) Cocaethylene (42 ng/mL) Ethanol (211 mg/dL)	Benzoylecgonine (40048.2 ng/mL) Cocaine (24458.8 ng/mL) Cocaethylene (3530.2 ng/mL)	Methylone Dimethylone Ethylone Benzoylecgonine Cocaethylene Cocaine 6-Monoacetylmorphine MDMA Morphine-Free
MS077	Ethanol (131 mg/dL)	-	Cocaine
MS078	-	Carboxy-THC (34.5 ng/mL) Ethylone (2046.5 ng/mL) Ethanol (244 mg/dL)	Ethylone (597.2 ng/mL) THC (23.2 ng/mL)
MS079	-	-	Ethylone (2481.2 ng/mL) THC (77 ng/mL)
MS080	-	-	Ethylone (246.3 ng/mL) THC (77 ng/mL)
MS081	-	Acetone (50 mg/dL)	ND
MS082	-	Fluoxetine (6820 ng/mL)	ND
MS083	-	Benzoylecgonine (448.9 ng/mL) Cocaine (<10 ng/mL) 2C-B (60 ng/mL)	Benzoylecgonine Cocaine
MS084	-	Dextromethorphan (<50 ng/mL) Carboxy-THC (>200 ng/mL)	THC (41.5 ng/mL)
MS085	-	-	THC (20.1 ng/mL)
MS086	-	ND	ND
MS087	ND	ND	ND
MS088	ND	ND	ND
MS089	Ethanol (35 mg/dL)	MDA (<50 ng/mL) MDMA (<50 ng/mL) Dextromethorphan (<50 ng/mL) Benzoylecgonine (957.3 ng/mL) Cocaine (<10 ng/mL)	Benzoylecgonine (1.2 ng/mL*)
MS090	-	Methamphetamine (79.4 ng/mL) Buprenorphine (>100 ng/mL) Norbuprenorphine (48.6 ng/mL) Carboxy-THC (>200 ng/mL) Benzoylecgonine (8398.6 ng/mL) Cocaine (22.0 ng/mL) Methylone (5102.1 ng/mL)	Methylone (1483.1 ng/mL) THC (44.9 ng/mL) Ethylone Benzoylecgonine Cocaine Lorazepam Methamphetamine

MS091	-	-	4-FA (281.6 ng/mL) THC (6.6 ng/mL) Methylone
MS092	-	Carboxy-THC (>200 ng/mL) Benzoylecgonine (7903.2 ng/mL) Cocaine (<10 ng/mL) Methylone (1813.1 ng/mL)	Methylone (251.5 ng/mL) THC (21.8 ng/mL) Benzoylecgonine Lorazepam
MS093	ND	ND	ND
MS094	THC-COOH (<5 ng/mL)	Carboxy-THC (7.0 ng/mL) Dextromethorphan (<50 ng/mL) Benzoylecgonine (25.2 ng/mL) Methylone (1016.6 ng/mL) Butylone (<1 ng/mL) Dimethylone (348.9 ng/mL)	Methylone (45.4 ng/mL) THC (<2 ng/mL)
MS095	-	-	ND
MS096	-	-	THC (140.1 ng/mL)
MS097	Ethanol (82 mg/dL)	Alpha-PVP (85.4 ng/mL) Ethanol (18 mg/dL)	Alpha-PVP
MS098	-	ND	ND
MS099	-	ND	ND
MS100	-	-	Methylone (4357.6 ng/mL) Ethylone (1351.3 ng/mL) Butylone (905.7 ng/mL) Alpha-PVP
MS101	-	-	Ethylone (423.4 ng/mL) THC (70.4 ng/mL) Methylone Butylone
MS102	-	-	Methylone (1870.7 ng/mL) Ethylone (1079.7 ng/mL) Butylone (175.0 ng/mL) Alpha-PVP
MS103	-	-	ND
MS104	-	-	MDMA
MS105	-	-	Methylone (7795.3 ng/mL) MDA MDMA
MS106	Tramadol (28 ng/mL) Ethanol (62 mg/dL)	Ethanol (33 mg/dL) Tramadol	ND

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MS107	THC (1.3 ng/mL) THC-COOH (48 ng/mL) Amphetamine (160 ng/mL) Alpha-PVP (10 ng/mL)	Amphetamine (23196.9 ng/mL) MDA (<50 ng/mL) PPA (155.2 ng/mL) Norketamine (<50 ng/mL) Dehydronorketamine (60 ng/mL) Carboxy-THC (>200 ng/mL) Methylone (22.6 ng/mL) Alpha-PVP (1474.1 ng/mL) Acetone (1 mg/dL)	Alpha-PVP (128.8 ng/mL) THC (111 ng/mL)
MS108	Amphetamine (150 ng/mL) THC (1.8 ng/mL) THC-COOH (52 ng/mL)	Amphetamine (17961.1 ng/mL) MDA (108.8 ng/mL) PPA (194.3 ng/mL) Carboxy-THC (>200 ng/mL) Benzoylecgonine (14.5 ng/mL) Methylone (112.4 ng/mL) Alpha-PVP (169.0 ng/mL) Acetone (2 mg/dL)	THC (60.9 ng/mL) Methylone Alpha-PVP
MS109	Alprazolam (8.3 ng/mL) THC (3.1 ng/mL) THC-COOH (82 ng/mL) Alpha-PVP (8.4 ng/mL)	Carboxy-THC (>200 ng/mL) Alpha-PVP (215.3 ng/mL)	Alpha-PVP (87.8 ng/mL) THC (207.3 ng/mL)
MS110	-	ND	ND
MS111	THC (2.3 ng/mL) THC-COOH (27 ng/mL) Alprazolam (16 ng/mL)	Carboxy-THC (157.9 ng/mL) Alprazolam (212.8 ng/mL) 1-OH Alprazolam (513.9 ng/mL) Oxazepam (25 ng/mL)	THC (3.8 ng/mL) Alpha-PVP
MS112	-	Norketamine (<50 ng/mL) Dehydronorketamine (120 ng/mL) Carboxy-THC (93.1 ng/mL)	-
MS113	-	Bupropion (230 ng/mL) Carboxy-THC (57.7 ng/mL)	ND
MS114	-	-	ND
MS115	-	Carboxy-THC (43.3 ng/mL)	ND
MS116	-	Carboxy-THC (70.7 ng/mL)	THC (10.7 ng/mL)
MS117	-	-	Methylone (196.2 ng/mL) Ethylone (350.8 ng/mL)
MS118	-	-	Ethylone (159.8 ng/mL) THC (2 ng/mL) Methylone
MS119	Methylone (7.1 ng/mL) Dimethylone (53.7 ng/mL) Ethanol (121 ng/mL)	Methylone (863.7 ng/mL) Dimethylone (1808.2 ng/mL) Ethanol (152 mg/dL)	Methylone Dimethylone

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MS120	Methylone (10.4 ng/mL) Dimethylone (65.6 ng/mL) Ethanol (123 mg/dL)	Methylone (987.6 ng/mL) Butylone (1.5 ng/mL) Dimethylone (2549.2 ng/mL) Ethanol (141 mg/dL)	Methylone Dimethylone
MS121	Ethanol (218 mg/dL)	-	ND
MS122	THC-COOH (<5 ng/mL) Ethanol (154 mg/dL)	Carboxy-THC (33.2 ng/mL) Ethylone (<1 ng/mL) Butylone (1.8 ng/mL) Alpha-PVP (2549.2 ng/mL) Ethanol (153 mg/dL)	ND
MS123	THC (3.9 ng/mL) THC-COOH (7.6 ng/mL) MDMA (<5 ng/mL)	Carboxy-THC (30.3 ng/mL) MDA (111.9 ng/mL) MDMA (696.5 ng/mL) Benzoylecgonine (16.9 ng/mL) Methylone (34.4 ng/mL) Acetone (2 mg/dL)	THC (10 ng/mL)
MS124	THC (1.4 ng/mL) THC-COOH (28 ng/mL) 7-Amino Clonazepam (40 ng/mL) Clonazepam (41 ng/mL) Methylone (362.3 ng/mL) Alpha-PVP (87 ng/mL) Ethanol (50 mg/dL)	Carboxy-THC (>200 ng/mL) Amphetamine (1348.3 ng/mL) MDA (1158.6 ng/mL) MDMA (5847.5 ng/mL) 7-Amino Clonazepam (743.5 ng/mL) Methylone (91093.3 ng/mL) Ethylone (<1 ng/mL) Dimethylone (57.9 ng/mL) Butylone (<1 ng/mL) 4-FA (7595.4 ng/mL) Alpha-PVP (>50000 ng/mL) Ethanol (23 mg/dL) Acetone (2 mg/dL)	Methylone (7388.1 ng/mL) Alpha-PVP (1301.6 ng/mL) THC (93 ng/mL) Amphetamine Clonazepam MDA MDMA
MS125	ND	Carboxy-THC (12.8 ng/mL)	THC (<2 ng/mL)
MS126	Oxycodone(Total) (61 ng/mL) THC-COOH (6.4 ng/mL)	Carboxy-THC (53.5 ng/mL) Oxycodone (>3000 ng/mL) Norquetiapine (1970 ng/mL) Quetiapine (30 ng/mL)	Oxycodone THC (26.6 ng/mL)
MS127	-	Carboxy-THC (>200 ng/mL) Benzoylecgonine (13973.7 ng/mL) Cocaine (32.9 ng/mL) Cocaethylene (38.5 ng/mL) Butylone (1059.6 ng/mL) Alpha-PVP (10.9 ng/mL) Ethanol (4 mg/dL)	Butylone (412.0 ng/mL) THC (21.5 ng/mL) Alpha-PVP Benzoylecgonine Cocaethylene Cocaine
MS128	Ethanol (35 mg/dL)	-	THC (15.4 ng/mL)

MS129	-	Carboxy-THC (169.8 ng/mL) Benzoylecgonine (>10000 ng/mL) Cocaine (<10 ng/mL) Cocaethylene (16.0 ng/mL) Ethanol (17 mg/dL)	THC (38.9 ng/mL) Benzoylecgonine Cocaethylene Cocaine
MS130	-	Methylphenidate (90 ng/mL) Carboxy-THC (18.6 ng/mL)	ND
MS131	-	-	THC (<2 ng/mL)
MS132	-	Dextromethorphan (<50 ng/mL) Alpha-PVP (8.4 ng/mL)	THC (<2 ng/mL) MDA
MS133	-	MDMA (<50 ng/mL) Dextromethorphan (<50 ng/mL) Oxycodone (50 ng/mL)	ND
MS134	-	ND	MDA
MS135	ND	Norquetiapine (340 ng/mL)	ND
MS136	Ethanol (84 mg/dL)	Amantadine (90 ng/mL) Ethanol (76 mg/dL)	ND
MS137	Ethanol (200 mg/dL)	Ethanol (197 mg/dL)	ND
MS138	-	-	ND
MS139	-	-	Methylone (792.5 ng/mL) THC (520.5 ng/mL) MDA MDMA Methamphetamine
MS140	-	-	ND
MS141	-	-	ND
MS142	-	-	Ethylone (41.6 ng/mL) THC (4 ng/mL) Alpha-PVP Benzoylecgonine Cocaine
MS143	-	MDA (13269.9 ng/mL) Carboxy-THC (<5 ng/mL) Benzoylecgonine (535.7 ng/mL) Cocaine (<10 ng/mL) Ethylone (145.3 ng/mL) Butylone (1.6 ng/mL) Ethanol (52 mg/dL)	-
MS144	-	Methamphetamine (<50 ng/mL) MDA (>2000 ng/mL) Benzoylecgonine (39.9 ng/mL) Cocaine (<10 ng/mL) Acetone (3 mg/dL)	-

MS145	-	Methamphetamine (<50 ng/mL) MDA (2742 ng/mL) MDMA (401.2 ng/mL) DMAA (65.9 ng/mL) Benzoylecgonine (122.4 ng/mL) Alpha-PVP (315.2 ng/mL) Dimethylone (9.4 ng/mL) Ethanol (121 mg/dL)	-
MS146	THC-COOH (28 ng/mL)	Carboxy-THC (128.8 ng/mL)	ND
MS147	THC (1.4 ng/mL) THC-COOH (25 ng/mL)	Carboxy-THC (318.8 ng/mL)	THC (<2 ng/mL)
MS148	Ethanol (48 mg/dL)	Ethanol (31 mg/dL)	ND
MS149	-	-	Ethylone (928.3 ng/mL) THC (10 ng/mL)
MS150	-	Desmethylsertraline (134 ng/mL) Sertraline (< 50 ng/mL)	ND
MS151	THC-COOH (44 ng/mL)	Carboxy-THC (818.3 ng/mL) Benzoylecgonine (2710 ng/mL)	THC (9.2 ng/mL) Benzoylecgonine
MS152	Ethanol (10 mg/dL)	Sertraline (241 ng/mL) Desmethylsertraline (471 ng/mL)	ND
MS153	-	-	ND
MS154	-	-	ND
MS155	THC (1.3 ng/mL) THC-COOH (18 ng/mL)	Carboxy-THC (122.4 ng/mL)	ND
MS156	-	ND	ND
MS157	Ethanol (22 mg/dL)	Ethanol (30 mg/dL)	ND
MS158	-	Carboxy-THC (16.0 ng/mL) MDMA (43 ng/mL) MDA (58 ng/mL)	ND
MS159	Ethanol (155 mg/dL)	Ethanol (146 mg/dL)	ND
MS160	Ethanol (107 mg/dL)	Ethanol (66 mg/dL) Chlorpheniramine (40 ng/mL) Pseudoephedrine (1790 ng/mL) PPA (260 ng/mL)	MDA
MS161	-	ND	ND
MS162	Ethanol (177 mg/dL)	-	ND
MS163	ND	ND	ND
MS164	-	-	THC (41.4 ng/mL)
MS165	-	-	THC (<2 ng/mL) MDMA
MS166	-	-	THC (265.8 ng/mL)
MS167	-	Carboxy-THC (39.2 ng/mL) Chlorpheniramine (20 ng/mL)	THC (10.6 ng/mL)

MS168	-	-	THC (30.3 ng/mL)
MS169	-	Carboxy-THC (36.5 ng/mL)	THC (119.2 ng/mL)
MS170	-	Ethanol (109 mg/dL)	ND
MS171	-	Ethanol (171 mg/dL)	ND
MS172	-	Carboxy-THC (73.9 ng/mL)	THC (21.4 ng/mL)
MS173	-	Carboxy-THC (142.2 ng/mL) Alprazolam (82 ng/mL) a-Hydroxyalprazolam (No Quant) Benzoylecgonine (35 ng/mL)	THC (14 ng/mL)
MS174	-	-	THC (5.2 ng/mL)
MS175	THC-COOH (5.3 ng/m) Ethylone (210.6 ng/mL)	Carboxy-THC (68.1 ng/mL) Ethylone (3318 ng/mL)	Ethylone (584.5 ng/mL)
MS176	-	Ethanol (44 mg/dL)	ND
MS177	-	Ethanol (71 mg/dL)	Ethylone
MS178	-	ND	ND
MS179	-	-	ND
MS180	-	ND	ND
MS181	-	Carboxy-THC (367.9 ng/mL)	THC (<2 ng/mL)
MS182	ND	ND	ND
MS183	-	-	ND
MS184	-	-	ND
MS185	-	Carboxy-THC (18.4 ng/mL) Dextromethorphan (90130 ng/mL) Doxylamine (1150 ng/mL)	ND
MS186	-	Ethanol (38 mg/dL)	ND
MS187	ND	ND	ND
MS188	-	Carboxy-THC (362.8 ng/mL) Ethylone (2994 ng/mL)	THC (107.8 ng/mL) Ethylone (1922 ng/mL)
MS189	-	Carboxy-THC (120.0 ng/mL) Ethylone (282 ng/mL)	THC (46.7 ng/mL) Ethylone (126 ng/mL)
MS190	-	-	ND
MS191	-	ND	ND
MS192	ND	ND	ND
MS193	-	Citalopram (150 ng/mL)	ND
MS194	-	-	THC (292.5 ng/mL) Benzoylecgonine
MS195	-	-	ND
MS196	-	-	THC (9 ng/mL)
MS197	-	-	ND
MS198	-	-	Benzoylecgonine Cocaethylene Cocaine
MS199	-	-	ND

MS200	-	ND	ND
MS201	-	-	ND
MS202	-	ND	ND
MS203	-	-	THC (16.7 ng/mL)
MS204	Ethanol (304 mg/dL)	Ethanol (190 mg/dL)	Cocaine
MS205	Ethanol (73 mg/dL)	Ethanol (52 mg/dL)	ND
MS206	-	Carboxy-THC (15.2 ng/mL)	ND
MS207	-	Carboxy-THC (77.5 ng/mL)	THC (2 ng/mL)
MS208	-	-	ND
MS209	-	Amphetamine (1110 ng/mL)	ND
MS210	Ethanol (27 mg/dL)	ND	ND
MS211	THC-COOH (11 ng/mL)	Carboxy-THC (61.9 ng/mL)	THC (2.6 ng/mL)
MS212	-	-	THC (9.2 ng/mL) MDA MDMA
MS213	Cocaine (130 ng/mL)	-	Benzoylecgonine Cocaine Ethylone
MS214	Benzoylecgonine (400 ng/mL)	-	Ethylone (377.3 ng/mL) Benzoylecgonine Cocaine
MS215	Ethanol (150 mg/dL)	Ethanol (101 mg/dL)	ND
MS216	Ethanol (66 mg/dL)	Ethanol (55 mg/dL)	ND
MS217	ND	Carboxy-THC (50.9 ng/mL)	ND
MS218	-	-	THC (143.5 ng/mL) Ethylone (63.1 ng/mL)
MS219	ND	ND	ND
MS220	ND	Carboxy-THC (26.8 ng/mL)	ND
MS221	-	-	ND
MS222	-	ND	ND
MS223	-	ND	ND
MS224	-	Carboxy-THC (183.0 ng/mL)	THC (44.3 ng/mL)
MS225	-	MDMA (2930 ng/mL) MDA (320 ng/mL) LSD (130 ng/mL) Norketamine (719 ng/mL) Dehydronorketamine (5581 ng/mL) Ketamine (244 ng/mL) Ethylone (540 ng/mL)	Ethylone (56.5 ng/mL) MDA MDMA
MS226	-	-	Ethylone (51.6 ng/mL) MDMA
MS227	Ethanol (31 mg/dL)	ND	ND
MS228	Ethanol (150 mg/dL)	-	ND

MS229	-	-	ND
MS230	-	ND	ND
MS231	-	ND	ND
MS232	-	-	ND
MS233	Ethanol (186 mg/dL)	Ethanol (104 mg/dL)	ND
MS234	-	Chlorpheniramine (100 ng/mL)	ND
MS235	-	-	ND
MS236	THC (1.3 ng/mL) THC-COOH (19 ng/mL) Benzoylecgonine (350 ng/mL)	Carboxy-THC (44.7 ng/mL) MDMA (990 ng/mL) MDA (80 ng/mL) a-hydroxyalprazolam (49 ng/mL) Cocaine (3650 ng/mL) Benzoylecgonine (51130 ng/mL) Cocaethylene (66 ng/mL) Ethylone (259 ng/mL)	Benzoylecgonine Cocaine
MS237	Ethanol (77 mg/dL)	Ethanol (88 mg/dL)	ND
MS238	-	-	ND
MS239	Ethanol (155 mg/dL)	Ethanol (168 mg/dL)	ND
MS240	Ethanol (132 mg/dL)	Ethanol (153 mg/dL)	ND
MS241	-	-	ND
MS242	-	Ethanol (60 mg/dL)	ND
MS243	ND	Carboxy-THC (156.2 ng/mL)	THC (4.8 ng/mL)
MS244	ND	Carboxy-THC (14.2 ng/mL) Cocaine (<10 ng/mL) Benzoylecgonine (2620 ng/mL) Cocaethylene (<10 ng/mL)	ND
MS245	-	Ethanol (28 mg/dL)	ND
MS246	MDMA (59 ng/mL) MDA (7.5 ng/mL) THC (1.3 ng/mL) THC-COOH (16 ng/mL)	Carboxy-THC (189.2 ng/mL) MDMA (16160 ng/mL) MDA (1790 ng/mL) Benzoylecgonine (89 ng/mL)	THC (<2 ng/mL) MDA MDMA
MS247	Cocaine (21 ng/mL) Benzoylecgonine (200 ng/mL) Ethanol (203 mg/dL)	Ethanol (182 mg/dL) Amphetamine (3810 ng/mL) Cocaine (90.6 ng/mL) Benzoylecgonine (14079 ng/mL) Cocaethylene (524 ng/mL)	Amphetamine Benzoylecgonine Cocaethylene Cocaine
MS248	ND	Carboxy-THC (109.4 ng/mL)	ND
MS249	-	Ethanol (22 mg/dL) Cocaine (84 ng/mL) Benzoylecgonine (22757 ng/mL) Cocaethylene (190 ng/mL)	Benzoylecgonine Cocaethylene Cocaine

MS250	Alprazolam (56 ng/ml) Benzoylecgonine (300 ng/mL) Ethanol (74 ng/mL)	Ethanol (36 mg/dL) Carboxy-THC (84.2 ng/mL) Phenobarbital (230 ng/mL) Alprazolam (420 ng/mL) a-Hydroxyalprazolam (1120 ng/mL) Cocaine (11549 ng/mL) Benzoylecgonine (155279 ng/mL) Cocaethylene (1858 ng/mL) Dextromethorphan (30 ng/mL) 3,4,5-Trimethoxycocaine (71 ng/mL) Doxylamine (200 ng/mL)	THC (<2 ng/mL) Alprazolam Benzoylecgonine Cocaine
MS251	-	Ethanol (20 mg/dL) Alprazolam (69 ng/mL) a-hydroxyalprazolam (100 ng/mL) Cocaine (314 ng/mL) Benzoylecgonine (16400 ng/mL) Cocaethylene (117ng/mL)	Benzoylecgonine Cocaine
MS252	-	-	THC (7.9 ng/mL) Ethylone (151.8 ng/mL)
MS253	THC-COOH (17 ng/mL) Ethanol (102 ng/mL)	Ethanol (70 mg/dL) Carboxy-THC (14.9 ng/mL)	THC (14.5 ng/mL)
MS254	Ethanol (25 ng/mL)	Ethanol (192 mg/dL)	THC (6.2 ng/mL)
MS255	-	ND	ND
MS256	-	Ethanol (38 mg/dL)	ND
MS257	-	Ethanol (179 mg/dL)	Ethylone (56.5 ng/mL)
MS258	-	-	ND
MS259	-	-	ND
MS260	-	-	THC (310.1 ng/mL) Benzoylecgonine Cocaethylene Cocaine
MS261	-	-	ND
MS262	-	-	ND
MS263	-	Ethanol (73 mg/dL)	ND
MS264	-	-	ND
MS265	-	Ethanol (141 mg/dL)	ND
MS266	THC (28 ng/mL) THC-COOH (87 ng/mL) THC-OH (10 ng/mL)	Carboxy-THC (99.4 ng/mL)	ND

MS267	-	Ethanol (22 mg/dL) Carboxy-THC (98.6 ng/mL) Alprazolam (200 ng/mL) a-Hydroxyalprazolam (210 ng/mL) Cocaine (13 ng/mL) Benzoylecgonine (111 ng/mL)	THC (32.7 ng/mL) Cocaine
MS268	ND	ND	ND
MS269	-	-	THC (24.7 ng/mL)
MS270	-	-	THC (76.8 ng/mL)
MS271	-	ND	ND
MS272	ND	ND	ND
MS273	-	-	Benzoylecgonine
MS274	-	-	Benzoylecgonine Methamphetamine
MS275	ND	-	THC (86 ng/mL)
MS276	-	Ethanol (52 mg/dL)	ND
MS277	-	-	ND
MS278	-	-	ND
MS279	Hydrocodone (28 ng/mL) Morphine (Total) (130 ng/mL)	Morphine (37300 ng/mL) Hydrocodone (2960 ng/mL) Hydromorphone (770 ng/mL) Oxycodone (210 ng/mL) Oxymorphone (820 ng/mL) Diphenhydramine (12520 ng/mL) Dihydrocodeine (295 ng/mL)	Hydrocodone Morphine Oxycodone
MS280	-	-	ND
MS281	-	ND	ND
MS282	-	-	ND
MS283	Cocaine (24 ng/mL)	Carboxy-THC (16.0 ng/mL) Cocaine (197 ng/mL) Benzoylecgonine (1615 ng/mL)	THC (<2 ng/mL) Ethylone Benzoylecgonine Cocaine
MS284	Cocaine (45 ng/mL) Benzoylecgonine (140 ng/mL) THC-COOH (11 ng/mL)	Carboxy-THC (253 ng/mL) Cocaine (160 ng/mL) Benzoylecgonine (15250 ng/mL)	THC (10.1 ng/mL) Benzoylecgonine Cocaine
MS285	-	Carboxy-THC (180.9 ng/mL) Cocaine (30 ng/mL) Benzoylecgonine (4402 ng/mL)	THC (<2 ng/mL) Benzoylecgonine Cocaine
MS286	ND	Naproxen (53400 ng/mL)	ND
MS287	ND	ND	ND
MS288	-	Amphetamine (250 ng/mL) Methamphetamine (1180 ng/mL)	Amphetamine Methamphetamine
MS289	-	-	THC (2.5 ng/mL)

MS290	-	-	THC (2 ng/mL)
MS291	-	-	THC (33.3 ng/mL)
MS292	-	-	THC (<2 ng/mL)
MS293	-	-	ND
MS294	-	Ethanol (82 mg/dL) Carboxy-THC (97.1 ng/mL)	ND
MS295	-	ND	ND
MS296	-	Ethanol (20 mg/dL) Ethylone (1610 ng/mL)	Ethylone (376.4 ng/mL)
MS297	-	-	Ethylone (341.7 ng/mL)
MS298	-	Ethanol (315 mg/dL)	ND
MS299	-	Ethanol (210 mg/dL)	ND
MS300	-	-	THC (16.8 ng/mL) Ethylone
MS301	Alprazolam (12 ng/mL)	-	THC (3.1 ng/mL)
MS302	-	-	THC (4 ng/mL)
MS303	-	Ethanol (56 mg/dL) Carboxy-THC (20.0 ng/mL)	THC (<2 ng/mL)
MS304	-	-	THC (2.7 ng/mL)
MS305	-	-	THC (70.6 ng/mL) MDA MDMA
MS306	-	-	THC (6.6 ng/mL)
MS307	-	-	ND
MS308	-	-	ND
MS309	Cocaine (21 ng/mL) MDMA (25 ng/mL) THC-COOH (33 ng/mL)	Carboxy-THC (468.6 ng/mL) MDMA (4120 ng/mL) MDA (190 ng/mL) Cocaine (2209 ng/mL) Benzoylecgonine (1066 ng/mL) Cocaethylene (15 ng/mL)	THC (446.6 ng/mL) Benzoylecgonine Cocaine MDMA
MS310	-	Ethylone (253 ng/mL)	Ethylone (503.1 ng/mL)
MS311	-	-	Ethylone (1552.2 ng/mL)
MS312	-	MDMA (1400 ng/mL) MDA (220 ng/mL)	ND
MS313	-	MDMA (730 ng/mL) MDA (90 ng/mL)	ND
MS314	-	Ethanol (21 mg/dL)	ND
MS315	Ethanol (25 mg/dL)	-	ND
MS316	-	ND	ND
MS317	-	ND	ND
MS318	-	-	THC (320.6 ng/mL)
MS319	Ethanol (104 mg/dL)	Ethanol (134 mg/dL)	ND

Appendix D

MS320	Benzoylecgonine (280 ng/mL) Cocaine (45 ng/mL) Cocaethylene (27 ng/mL) Ethanol (157 mg/dL)	Ethanol (184 mg/dL) Cocaine (6170 ng/mL) Benzoylecgonine (18000 ng/mL) Cocaethylene (2830 ng/mL)	Benzoylecgonine Cocaethylene Cocaine
MS321	-	Ethanol (144 mg/dL)	THC (2.7 ng/mL) Ethylone
MS322	ND	ND	ND
MS323	-	Ethanol (115 mg/dL) Carboxy-THC (31.8 ng/mL) Ethylone (7130 ng/mL)	THC (66.5 ng/mL) Ethylone (4105 ng/mL) MDMA
MS324	-	Ethanol (37 mg/dL) Carboxy-THC (16.9 ng/mL) MDMA (1750 ng/mL) MDA (190 ng/mL) Ethylone (2614 ng/mL)	THC (8.1 ng/mL) Ethylone (114.5 ng/mL) MDMA
MS325	-	-	THC (84 ng/mL) Ethylone (169.9 ng/mL) Dextromethorphan MDA MDMA
MS326	-	-	THC (45 ng/mL) Ethylone (513.1 ng/mL) Benzoylecgonine Cocaine MDMA
MS327	THC-COOH (34 ng/mL) Ethanol (151 mg/dL)	Ethanol (187 mg/dL) Carboxy-THC (32.2 ng/mL)	THC (7.5 ng/mL)
MS328	-	-	THC (147.8. ng/mL) Ethylone (188.4 ng/mL) MDA MDMA
MS329	Dimethylone (10.7 ng/mL) Ethylone (212.9 ng/mL) Methylone (9.9 ng/mL)	Carboxy-THC (79.0 ng/mL) Orphenadrine (3500 ng/mL) Chlorpheniramine (650 ng/mL) Methylone (1901 ng/mL) Butylone (2327 ng/mL) Ethylone (>57,000 ng/mL) Dimethylone (1060 ng/mL) Doxylamine (2280 ng/mL) 2C-I (0.29 ng/mL) 25I-NBOMe (0.25 ng/mL) 25I-NBOH (0.71 ng/mL)	Methylone (40.3 ng/mL) Ethylone (728.5 ng/mL) Dimethylone
MS330	ND	Carboxy-THC (42.0 ng/mL)	THC (<2 ng/mL)

MS331	-	-	THC (<2 ng/mL)
MS332	Alprazolam (34 ng/mL) MDMA (270 ng/mL) MDA (26 ng/mL) Ephedrine (29 ng/mL) Ethanol (24 mg/dL)	Carboxy-THC (7.6 ng/mL) MDMA (9810 ng/mL) MDA (660 ng/mL) Ephedrine (1220 ng/mL) PPA (90 ng/mL) Alprazolam (110 ng/mL) a-Hydroxyalprazolam (230 ng/mL) N,N-Dimethyltryptamine (61 ng/mL) Benzoylecgonine (50 ng/mL)	THC (511.8 ng/mL) Ethylone Cocaine MDA MDMA
MS333	-	-	ND
MS334	-	MDMA (301000 ng/mL) MDA (23240 ng/mL) Cocaine (113 ng/mL) Benzoylecgonine (19000 ng/mL) Naproxen (693 ng/mL) Methylone (286 ng/mL) Ethylone (11971 ng/mL)	Ethylone (199.2 ng/mL) Benzoylecgonine Cocaine MDA MDMA
MS335	-	-	THC (4.2 ng/mL) Ethylone
MS336	-	-	THC (10.6 ng/mL) Ethylone (154 ng/mL) Benzoylecgonine Cocaine
MS337	-	-	THC (2 ng/mL) Ethylone (319.9 ng/mL) Benzoylecgonine
MS338	-	Ethanol (83 mg/dL) Ketamine (<25 ng/mL) Norketamine (<25 ng/mL) Dehydronorketamine (52 ng/mL)	ND
MS339	-	-	ND
MS340	-	Ethanol (212 mg/dL) Amphetamine (1080 ng/mL)	THC (17.8 ng/mL) Amphetamine
MS341	-	Ethanol (161 mg/dL)	ND
MS342	-	Ethanol (65 mg/dL)	ND
CWD001	-	-	Ethylone
CWD002	-	-	ND
CWD003	-	-	Ethylone (579 ng/mL)
CWD004	-	-	Ethylone
CWD005	-	-	Ethylone (57.4 ng/mL) MDA MDMA

CWD006	-	-	THC (95.2 ng/mL)
CWD007	-	-	THC (16.1 ng/mL)
CWD008	-	-	ND
CWD009	-	-	ND
CWD010	-	-	ND
CWD011	-	-	MDMA
CWD012	-	-	ND
CWD013	-	-	Ethylone
CWD014	-	-	THC (9.1 ng/mL)
CWD015	-	-	THC (5.9 ng/mL)
CWD016	-	-	THC (5.9 ng/mL)
CWD017	-	-	In Screen: Citalopram/Escitalpram THC (<2 ng/mL)
CWD018	-	-	ND
CWD019	-	-	ND
CWD020	-	-	THC (157.4 ng/mL)
CWD021	-	-	ND
CWD022	-	-	ND
CWD023	-	-	ND
CWD024	-	-	ND
CWD025	-	-	THC (3.1 ng/mL)
CWD026	-	-	THC (208.1 ng/mL)
CWD027	-	-	ND
CWD028	-	-	Ethylone (46.5 ng/mL)
CWD029	-	-	Ethylone
CWD030	-	-	THC (4.4 ng/mL)
CWD031	-	-	THC (5.9 ng/mL)
CWD032	-	-	In Screen: Ketamine/Norketamine
CWD033	-	-	THC (857 ng/mL) Ethylone Benzoylecgonine Cocaine MDMA
CWD034	-	-	MDMA
CWD035	-	-	THC (<2 ng/mL) Ethylone
CWD036	-	-	ND
CWD037	-	-	Ethylone
CWD038	-	-	Ethylone (193.6 ng/mL) THC (23.5 ng/mL)

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CWD039	-	-	THC (23.5 ng/mL) Amphetamine Benzoylecgonine Cocaethylene Cocaine
CWD040	-	-	THC (<2 ng/mL)
CWD041	-	-	ND
CWD042	-	-	THC (96.3 ng/mL) MDA MDMA
CWD043	-	-	THC (17.9 ng/mL)
CWD044	-	-	ND
CWD045	-	-	THC (8.7 ng/mL)
CWD046	-	-	THC (6.8 ng/mL)
CWD047	-	-	ND
CWD048	-	-	THC (155.9 ng/mL)
CWD049	-	-	THC (31.6 ng/mL)
CWD050	-	-	ND
CWD051	-	-	THC (29.5 ng/mL)
CWD052	-	-	THC (24.1 ng/mL)
CWD053	-	-	THC (14.6 ng/mL)
CWD054	-	-	THC (96.5 ng/mL)

A dash (-) means no biological specimen of that type provided. ND = none detected.