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Development of Improved Extraction/Purification Methods and Comprehensive Screening/Confirmation by LC-QqQ-MS Analysis for Novel Psychoactive Substances

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FLORIDA INTERNATIONAL UNIVERSITY

Miami, Florida

DEVELOPMENT OF IMPROVED EXTRACTION/PURIFICATION METHODS AND
COMPREHENSIVE SCREENING/CONFIRMATION BY LC-QqQ-MS ANALYSIS
FOR NOVEL PSYCHOACTIVE SUBSTANCES

A dissertation submitted in partial fulfillment of

the requirements for the degree of

DOCTOR OF PHILOSOPHY

in

CHEMISTRY

by

Ashley Nicole Kimble

2019

To: Dean Micheal R. Heithaus
College of Arts, Sciences and Education

This dissertation, written by Ashley Nicole Kimble, and entitled Development of Improved Extraction/Purification Methods and Comprehensive Screening/Confirmation by LC-QqQ-MS Analysis for Novel Psychoactive Substances, having been approved in respect to style and intellectual content, is referred to you for judgment.

We have read this dissertation and recommend that it be approved.

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Date of Defense: June 10, 2019

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Florida International University, 2019

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ABSTRACT OF THE DISSERTATION

DEVELOPMENT OF IMPROVED EXTRACTION/PURIFICATION METHODS AND
COMPREHENSIVE SCREENING/CONFIRMATION BY LC-QqQ-MS ANALYSIS
FOR NOVEL PSYCHOACTIVE SUBSTANCES

by

Ashley Nicole Kimble

Florida International University, 2019

Miami, Florida

Professor Anthony P. DeCaprio, Major Professor

The presence of novel psychoactive substances (NPS) in forensic casework poses major difficulties for detection, since there are many structural variations of NPS circulating in the street market. There are currently no comprehensive screening/confirmatory/quantitation methods available that encompass the majority of NPS encountered in forensic toxicology. A major issue faced with developing such a method is that full validation is extremely time consuming. The use of a liquid chromatography triple quadrupole tandem mass spectrometry (LC-QqQ-MS/MS) method makes the detection of a large number of NPS possible because of high selectivity and sensitivity.

This research included four main tasks: 1) development of a dynamic multiple reaction monitoring (dMRM) LC-QqQ-MS method for 800+ NPS, 2) validation of the dMRM method for screening and confirmation of 800+ NPS using a series of mixtures of non-coeluting standards, 3) comparison and optimization of NPS extraction methods for

urine and whole blood, and 4) screening of spiked and authentic specimens to determine the real-world potential of the dMRM method.

Validation was completed for the parameters of selectivity, limit of detection (LOD), limit of quantitation (LOQ), carry over, linearity, bias, precision, freeze-thaw stability, and matrix effects. A method that ultimately included a total of 729 compounds was validated with LOD and LOQ in the pg/mL range. The research presented here implements the largest validated method of its kind for NPS with capabilities as a screening method for NPS in urine and whole blood and as a confirmatory method in urine.

Several extraction methods were also compared to determine their efficacy for the extraction of NPS from urine and whole blood. These included dilute- and crash-and-shoot, online and classical solid phase extraction, and QuEChERS. Techniques were compared for elimination of matrix effects, recovery, process efficiency, time, and cost.

Through the analysis of blind spiked and authentic specimens, the applicability of the validated method as a screening and confirmatory method was successfully demonstrated. The method developed in this project will aid in reliable identification of NPS in clinical and forensic toxicological samples. Additionally, this work provided data to improve the reliability of extraction of NPS from biological matrices.

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LIST OF ABBREVIATIONS, ACRONYMS, AND SYMBOLS

AF	Ammonium Formate
ANOVA	Analysis of Variance
BE	Bond Elut
CB1	Cannabinoid Type 1 Receptor
CB2	Cannabinoid Type 2 Receptor
CDC	Center for Disease Control and Prevention
CE	Collision Energy
CI	Chemical Ionization
CSA	Controlled Substance Act
CV	Coefficient of Variation
d-SPE	Dispersive SPE
DEA	Drug Enforcement Administration
DFC	Drug Facilitated Crime
dMRM	Dynamic Multiple Reaction Monitoring
DMSO	Dimethyl sulfoxide
DUI	Driving Under the Influence
EC	Endocannabinoids
EC C18	Encapped C18
EDTA	Ethylenediaminetetraacetic acid
EI	Electron Ionization
EIC	Extracted Ion Chromatogram

ELISA	Enzyme Linked Immunosorbent Assay
EMIT	Enzyme Multiplied Immunoassay Technique
ESI	Electrospray Ionization
FA	Formic Acid
FIA	Flow Injection Analysis
GC-MS	Gas Chromatography Mass Spectrometry
H ₂ O	Water
HCl	Hydrochloric Acid
HPLC	High Performance Liquid Chromatography
HRMS	High Resolution Mass Spectrometry
HSD	Honestly Significant Difference
IS	Internal Standard
LC-MS	Liquid Chromatography Mass Spectrometry
LLE	Liquid-Liquid Extraction
LOD	Limit of detection
LOQ	Limit of Quantitation
<i>m/z</i>	Mass to Charge Ratio
ME	Matrix Effects
MeCN	Acetonitrile
MeOH	Methanol
mg	Milligram
mL	Milliliter
MRM	Multiple Reaction Monitoring

MS	Mass Spectrometry
MS/MS	Tandem Mass Spectrometry
NaCl	Sodium Chloride
ng	Nanogram
NIST	National Institute of Standards and Technology
NPS	Novel Psychoactive Substances
OSAC	Organization of Scientific Area Committees
OTC	Over the Counter
PCP	Phencyclidine
PE	Process Efficiency
PP	Protein Precipitation
ppb	Parts Per Billion
ppm	Parts Per Million
PSA	Primary Secondary Amine
QC	Quality Control
QqQ	Triple Quadrupole Mass Spectrometry
QTOF	Quadrupole Time-of-Flight Mass Spectrometry
QuEChERS	quick, easy, cheap, effective, rugged, and safe
RE	Recovery
RP	Reversed Phase
RT	Retention Time
SAMHSA	Substance Abuse and Mental Health Services Administration
SIM	Selected Ion Monitoring

SPE	Solid Phase Extraction
SRM	Selected Reaction Monitoring
SWGTOX	Scientific Working Group for Forensic Toxicology
THC	Δ^9 -Tetrahydrocannabinol
TIC	Total Ion Chromatogram
UPLC	Ultra Performance Liquid Chromatography
Δc	Change in Concentration
μg	Microgram
μL	Microliter

1. INTRODUCTION

1.1 Statement of the Problem

Novel psychoactive substances (NPS) have been a global health hazard for the past several decades. Novel psychoactive substances are structural alterations of controlled substances created to evade drug law. There have been reported fatal overdoses that can be attributed to NPS, especially from synthetic cannabinoids and opioids.¹⁻⁴ Novel psychoactive substances are difficult to control and detect in biological fluids, because of their constantly changing structures introduced by illicit manufacturers as current drugs become scheduled and illegal to possess. Drugs of abuse are scheduled according to their unique chemical structure, therefore every small structural alteration results in a compound no longer being regulated by controlled substance laws.⁵ Changes to structure can be as small as the addition or removal of a functional group or single atom, such as a halogen. Consequently, there are practically endless structural possibilities for NPS. Such derivatives can have extremely varied pharmacological effects, ranging from minimal effect to severe toxicity.^{2,6}

Many screening methods used in clinical and forensic toxicology detect compounds on the basis of their structure or specific functional groups. Since screening methods tend to be structure-specific, NPS can be missed during screening, resulting in false negatives. In a forensic or clinical setting, if a sample is wrongly reported as negative it is possible that the sample will be discarded, making it impossible to retest the sample with advanced methodology. False negatives are especially problematic when the results of these tests are being used for treatment and potentially determining cause of death. One of the biggest issues faced by law enforcement in terms of detecting NPS is that many manufacturers

have different structures waiting for distribution as soon as an existing NPS structure becomes known, scheduled, and detectable.⁷ Consequently, it is difficult for clinical and forensic toxicology laboratories to detect NPS as new ones become available.

A possible solution for the detection of NPS involves the creation of comprehensive libraries and databases containing chromatographic and mass spectral data for individual NPS entities. When combined with libraries and databases, comprehensive targeted screening and confirmatory methods have the potential to detect large numbers of NPS in clinical and forensic toxicology. Currently, there are a number of libraries for gas chromatography (GC) generated using electron ionization (EI) mass spectrometry (MS). Additionally, there exist libraries for liquid chromatography (LC) using electrospray ionization (ESI) MS, but these lack the comprehensiveness and standardization associated with existing GC libraries.^{8,9} Even though such MS libraries exist, many of them are theoretical (*i.e.*, determined by calculations of fragmentation rather than using actual reference standards to determine fragmentation) or contain few to no NPS. The majority of forensic toxicological laboratories have in place GC and/or LC-MS methods capable of detecting typical drugs of abuse and other compounds commonly found in their casework. In recent years, many such laboratories have also begun including NPS, but the number of NPS entities included in these types of methods is generally only a small representation of the sheer number of NPS that are potentially available.¹⁰ Clinical and forensic toxicology laboratories struggle to identify NPS in a timely and reliable manner. Misidentification can lead to major health epidemics if, for example, a potent NPS is not detected until after there have been multiple overdoses in a specific area. Research has been done in order to combat

these issues, but more needs to be done before clinical and forensic toxicology laboratories can properly detect NPS as they are produced and released to the illicit market.

In recent decades, NPS have become a major global public health issue, especially in the United States, Europe, and China.^{11,12} Specifically, in the United States synthetic opioids, especially fentanyl derivatives, are contributing to the opioid epidemic. Commonly used opioids and a number of novel derivatives have been the cause of death of thousands in the U.S. over the past few years. Not only are NPS concerning to the forensic toxicology communities, they are also a health hazard. Many NPS are first detected in Europe before being distributed to the U.S. Novel psychoactive substances are relatively easy to obtain since they can often be purchased via the internet. The internet has made the sale and purchasing of NPS easier than it would have been in the past. There is generally little pharmaceutical information available for the majority of NPS, which leads to the risk of increased overdoses and toxicity.¹³ Many consumers falsely believe that “legal” means safer, which is not the case for many NPS, further worsening their health hazard.¹³ The health concerns revolving around NPS need to be combatted, starting with identification in clinical and forensic toxicology. Being able to detect NPS quickly and reliably will aid in scheduling, further research, and informing the public of their hazards. Consequently, the development of screening and confirmatory methods for the detection of a wide variety of NPS will aid clinical and forensic toxicology laboratories in the timely and reliable detection of such harmful substances, helping to prevent or manage further epidemics.

1.2 Rationale for Research

Many toxicological laboratories use immunoassays for initial drug screening, which are robust when used for the detection of common drugs of abuse but can be problematic when working with NPS. Immunoassays are capable of detecting drug compounds through an antigen-antibody interaction which relies on the compound's structure. As a result of the structure relation requirement, issues arise when trying to screen for NPS, since they exhibit structural differences as compared to common drugs of abuse. Screening methods using LC and GC MS are more adaptable to the structural changes that NPS undergo than immunoassays. Analytical methods are also routinely used in forensic toxicological laboratories.⁹ However, most commonly these methods function based on library matches or a targeted method. Many of these methods and/or libraries only contain a small set of NPS, and some methods contain NPS that are no longer seen in modern case work. In addition, even though screening methods are available for some NPS, very few of those methods have been validated and are capable of quantitative results.

A similar issue to the detection of NPS arises when trying to extract NPS from biological matrices. Extraction methods for common drugs of abuse are well established in clinical and forensic toxicological laboratories. However, it is not always possible to use those same methods for the majority of NPS compounds. Many extraction methods rely on the structure and chemical properties of the compound to successfully extract the analyte of interest from its biological matrix. Since NPS undergo structural alterations, some of these methods may no longer be able to reliably separate the analyte of interest from the matrix. It is important to have extraction methods capable of isolating NPS from biological matrices and ensuring that they are not discarded as waste during the extraction process.

1.3 Significance of Study

The current research is designed to be applicable to forensic science, clinical and forensic toxicology, and law enforcement. The research described here has revolved around the development and validation of a comprehensive dynamic multiple reaction monitoring (dMRM) method for screening and confirmation of hundreds of NPS. The validated method was then used to evaluate the effectiveness of different extraction methods for NPS and to determine statistically significant differences among the methods. The ultimate goal is to implement optimized extraction approaches and a validated analytical method into forensic toxicology laboratories to aid in the timely and reliable detection of NPS in case samples.

The NPS to be included in the database generated for the project were determined by researching published articles, forensic case work reports, drug user blogs/forums, and overdose reports to ensure that the NPS being included were still in use and relevant for clinical and forensic toxicological samples. In addition to common NPS, the database also includes common adulterants, to ensure that the method is capable of differentiating the NPS from them. The research presented here was divided into four major tasks.

1.3.1 Task 1 – *Creation of a dMRM database and screening/confirmatory method*

This task was the foundation for all the tasks to come after. Up to 10 precursor-product ion transitions were collected for all NPS to be included in the final analytical method. Once transitions were established, all compounds were analyzed to determine retention times in order to create a dMRM method capable of screening for over 800 NPS.

1.3.2 Task 2 - *Validation of the dMRM screening/confirmatory method using a mixture approach*

After method development was finalized, full method validation was completed using a set of defined mixtures of non-coeluting NPS in order to decrease the time required to complete the validation. Method validation was performed following established toxicological guidelines. The parameters validated for included linearity, limit of detection, limit of quantitation, carry over, bias, precision, matrix effect, and freeze-thaw-stability.

1.3.3 Task 3 - *Evaluation and optimization of NPS extraction/purification methods*

The third portion of the research was completed by comparing crash-/dilute-and-shoot, QuEChERS (Quick, Easy, Cheap, Effective, Rugged, and Safe), online SPE, and classical SPE to determine which protocols have statistically significant benefits over the others for the extraction of NPS from urine and whole blood. Comparison was done using drug recovery, elimination of matrix effects, and improved process efficiency. Even though extraction methods for common drugs of abuse have been thoroughly studied, extraction methods for NPS have not been comprehensively evaluated. Extraction methods were compared and then optimized to ensure that the ideal conditions were used for each method.

1.3.4 Task 4 – *Analysis of blind spikes and authentic specimens*

To apply the current research to actual casework samples, blind spiked urine and whole blood were analyzed quantitatively and qualitatively. Blind spiked samples were used to ensure that the validated method is capable of correctly identifying multiple compounds

per sample in different matrices. Additionally, blind spikes were used to test the effectiveness of extraction methods used throughout the present research. Blind spikes were treated identically to how case samples would be prepared and tested. Finally, authentic urine specimens were also collected and tested using the validated method to identify the presence of NPS and to ensure that there were no interferences by common drugs of abuse and medications that were likely to be present in authentic samples.

2. LITERATURE REVIEW

2.1 Novel Psychoactive Substances

Novel psychoactive substances (NPS) have been gaining popularity over the past few decades by consumers, sellers, and manufacturers.¹⁴ Novel psychoactive substances are also known as “legal highs,” “designer drugs,” and “spice.” According to the Controlled Substances Act (CSA) passed in 1970, drugs of abuse are scheduled on the basis of their structure, pharmacological effect, potential for abuse, and accepted medical uses.¹⁵ These compounds can fall under one of five schedules. Schedule V compounds have a very low potential for abuse and many accepted medical uses. Schedule IV and III substances have a low potential for abuse and a moderate potential for abuse, respectively. Schedule I and II compounds have high potential for abuse and no or limited medical use, respectively. Novel psychoactive substances are specifically created to evade drug laws, as a consequence of their structural differences from common, scheduled drugs of abuse. These structural changes can be as simple as the addition of a methyl group, a small change that renders the compound novel and, therefore, unscheduled. Prime examples of this are the NBOMe compounds, which all have the same base structure but with the simple addition of a halogen atom that creates a new compound that is no longer scheduled.¹⁶

An NPS is a compound that is structurally and pharmacologically “substantially similar” to a Schedule I or II compound. The Drug Enforcement Administration (DEA) has been making an effort to schedule NPS through regulations such as The Federal Analog Act passed in 1986, which stated that new compounds can be scheduled if they are proven to be structurally and pharmacologically similar to a Schedule I or II compound. Although the act was helpful, proving structural and pharmacological similarity can be difficult. Not

all NPS have the same structural backbone as the common drugs of abuse to which they are designed to have similar pharmacological effects. A prime example of this are the synthetic cannabinoids. The structures of many synthetic cannabinoids vary greatly from that of Δ^9 -tetrahydrocannabinol (THC), which is the active component in marijuana. Synthetic cannabinoids can have extremely varied structural characteristics and are divided into separate classes within the general class. Some of these subclasses include cyclohexylphenols, naphthoylindoles, phenylacetylindoles, and indole carboxylates.¹⁷

There are efforts for temporary scheduling of NPS that are capable of leading to permanent scheduling within two years and adding an additional 12-month extension to continue research efforts. It is generally very difficult to monitor the distribution of NPS, since they are often sold online under the label of “not for human consumption.” It is not uncommon for NPS to be developed for research purposes but then diverted for illicit use, with their potential for abuse discovered at a later time.¹⁸ There are published books and online forums that give step-by-step instructions on the synthesis of different NPS, which only makes it easier for illicit drug manufacturers. Unfortunately, as a result of all the resources available to clandestine laboratories, it is difficult for entities such as the DEA to act proactively to schedule NPS.⁵

The speed at which drug manufacturers are able to place structurally different NPS on the market also makes it very difficult for forensic toxicologists to detect all of the different possible compounds that can be found in a specimen. New compounds that are found on the market and not detectable by current methods can then lead to false negatives, which is undesirable from a clinical and forensic viewpoint. It is important to have a method capable of detecting the majority of NPS that are available to consumers. Another issue that

forensic laboratories face when screening for NPS is that it is impossible to develop and validate methods as quickly as manufacturers can create new structures.

2.2 Classes of Novel Psychoactive Substances

There are a wide variety of NPS that have been recorded in literature and which can be placed into various drug classes depending on structure and pharmacological effects. Novel psychoactive substances can generally be classified according to the following structural categories; benzodiazepines, cathinones, phenethylamines, synthetic cannabinoids, synthetic opioids, and tryptamines.⁶ Different classes of NPS need to be treated differently for analysis depending on the structure and the chemical properties of their functional groups. For many NPS, the mechanism of action and pharmacological effects are unknown. The lack of understanding around NPS poses a health risk, since people are ingesting compounds that are not well understood. It is not uncommon for NPS to be more potent than many common drugs of abuse, contributing further to their status as health hazards.⁴

All drug classes of NPS are of public health concern. There are reported overdoses and in some cases fatalities for the majority of them in the past decade in the U.S. and around the world.^{19,20} There is typically little to no reliable pharmacological information available for these compounds and many of their mechanisms of actions are not well understood. For example, there have been numerous fatalities due to NBOMes, which are part of the phenethylamine class of NPS.^{16,21,22} Synthetic benzodiazepines have also been detected in forensic cases, with the most common compounds being flubromazolam and flubromazepam. Often, illicit benzodiazepines are seen in cases in combination with THC and amphetamine.²³

Three of the most prevalent classes of NPS are synthetic cathinones, synthetic cannabinoids, and synthetic opioids. Fatalities involving these drug classes have been reported all over the world, some of which can be considered to be epidemics. There have been cases of mass fatalities for all three of these categories in the U.S. alone. The phenomenon of increased potency of some of these compounds as compared to typical drugs of abuse is not well appreciated by users, further leading to overdoses and related fatalities. The most extensive research on NPS has been conducted on these three classes, resulting in a number of review articles focusing on use, mechanism of action, structures, and pharmacological effects.²⁴⁻²⁶

2.2.1 Synthetic Cathinones

Synthetic cathinones, also referred to as “bath salts,” have been abused in the U.S. and around the world for many years and are still being identified in clinical and forensic case samples.^{27,28} Synthetic cathinone abuse can be seen in both impaired driving cases and fatal intoxications.²⁹ Synthetic cathinones can vary considerably in structure, and, because of their varied structures, they can also differ in mechanism of action and pharmacological effects.³⁰ Synthetic cathinones act upon the monoamine transporters for dopamine, noradrenaline, and serotonin.³¹ As a result of variable structures of synthetic cathinones, their affinity for the transporters and ability to inhibit reuptake of these neurotransmitter molecules can vary greatly. These differences can lead to complex combinations of dopaminergic, adrenergic, and/or serotonergic effects in users.³² These effects contribute to the stimulant and mood-altering feelings associated with synthetic cathinone abuse.

As with many NPS, there is little to no pharmacological information available on synthetic cathinones. The pharmacology of synthetic cathinones is not well understood,

however, synthetic cathinones can exhibit similar effects to amphetamines. Side effects from synthetic cathinone use in high doses can include hallucinations, delirium, hyperthermia, and tachycardia. Chronic users can exhibit extreme agitation and violent behavior associated with “excited delirium.”³¹ Additional side effects can include dehydration, muscle damage, and organ failure. Heavy synthetic cathinone abuse in some cases can lead to death.

Detecting synthetic cathinones in human specimens poses numerous difficulties. For example, it is known that cathinones are not stable in plasma.³³ There are issues associated with the quantitative repeatability of synthetic cathinone test results. The stability and changes in concentration that can occur during storage are not well understood for synthetic cathinones, leading to issues with detection and quantification.³⁴ Another issue with analysis is that synthetic cathinones can undergo *in situ* degradation when analyzed using GC-MS, because of their low boiling point and thermal instability.³⁵ Synthetic cathinones need to be treated properly to ensure that they are not degraded during sample preparation or analysis.

2.2.2 Synthetic Cannabinoids

There have been findings of synthetic cannabinoids presenting much higher potencies than THC, which can lead to an increased number of overdose cases.³⁶ Synthetic cannabinoids are considered dangerous and the Center for Disease Control and Prevention (CDC) has posted warnings on their website suggesting that people under no circumstances use anything they purchased after March, 2018 because of recorded cases of extreme bleeding after use of synthetic cannabinoids.³⁷ Synthetic cannabinoids can be sprayed onto plant material and smoked, vaped from a liquid form, or used in different foods and

consumed orally. They can be found in convenience stores, but more often are purchased online.⁷

Not all synthetic cannabinoids were developed for illicit distribution; many were developed as part of legitimate scientific research. Some prime examples are the JWH compounds, which were discovered in the laboratory of John W. Huffman at Clemson University. The JWH compounds were synthesized to study their reactions with cannabinoid receptors in the brain. The initial research was published in 1998 however, JWH 018 was not found being used as an alternative to cannabis until 2008.^{18,38}

Synthetic cannabinoids can be subcategorized into several different classes determined by their structure. These subclasses include, but are not limited to, cyclohexylphenols, naphthoylindoles, benzoylindoles, phenylacetylindoles, alkoylindoles, indole carboxylates, indole carboxamides, and indazole carboxamides.¹⁷ Cyclohexylphenol cannabinoids are bicyclic derivatives of classical cannabinoids exhibiting the most similar structure to THC.¹⁷ Naphthoylindoles are considered to be the “first generation” of synthetic cannabinoids and were originally identified in herbal substances. The other subclasses are newer synthetic cannabinoids that arose from changing the naphthoyl moiety with varying aromatic and non-aromatic groups. There are practically endless possibilities of structures for synthetic cannabinoids to exhibit.

The cannabinoid system in the human body has naturally occurring neurotransmitters known as endocannabinoids (EC). Many synthetic cannabinoids act on the same receptors as THC, however there are hundreds of possible structures, some that vary greatly from THC. Similar to natural cannabinoids, synthetic cannabinoids compete with endogenous EC at CB receptor sites. The most common receptors in the cannabinoid system are CB₁

and CB₂.³⁹ The CB₁ and CB₂ receptors are located in different areas of the body and responsible for different effects once activated. The CB₁ receptor is expressed primarily in the brain and is associated with the psychotropic effects of THC.³¹ In contrast, the CB₂ receptor is primarily a peripheral receptor expressed in the immune, gastrointestinal, and other organ systems, although recent work has also reported the presence of CB₂ receptors in the brain. As a result of the complexity of the cannabinoid system in the body it is difficult to determine the targeted receptor and clinical effects of synthetic cannabinoids.

Synthetic cannabinoids exhibit many effects similar to commonly used cannabis products, although there are some differences. Physical effects caused by synthetic cannabinoids can include, but are not limited to, tachycardia, anxiety, hallucinations, acute kidney injury, convulsions, and psychosis.^{31,40} Synthetic cannabinoids have been associated with severe toxicity and deaths by consumers leading to a number of mass intoxication reports in the United States between 2013 and 2015.¹⁷ An example of an outbreak occurred in a small radius in New York City involving 33 intoxications due to AMB-FUBINACA.⁴¹

Synthetic cannabinoids can be metabolized into phase I and phase II metabolites. The metabolism of some synthetic cannabinoids has been studied and metabolites are readily found in clinical and forensic toxicological samples when testing urine.⁴² In fact, it is common to only detect metabolites of synthetic cannabinoids in urine rather than detecting the parent compound. Therefore, it is important that methods designed for the detection of synthetic cannabinoids also includes metabolites.

2.2.3 Synthetic Opioids

Since 2013 there has been an opioid crisis in the United States and synthetic opioids, especially fentanyl derivatives, are part of the epidemic.⁴³ Synthetic opioids are often much cheaper than heroin and other opioids, which is a major factor as to why they show up unknown to the consumer.⁴⁴ Not only are synthetic opioids inexpensive, they are also easily purchased online anonymously from countries such as China. Illicit drug sellers will purchase synthetic opioids online and, unknown to their customers, they lace heroin with it to make even more of a profit. It is important to realize that fentanyl, carfentanil, and other fentanyl derivatives are much more potent than other opiates and opioids like heroin and morphine.^{43,45} The fact that many fentanyl derivatives are so potent has led to an overabundance of synthetic opioid related overdoses and deaths. A large part of the opioid crisis revolves around drug users not knowing that heroin has been laced with more potent synthetic opioids, leading to fatalities.⁴⁶ During 2013 in Rhode Island, Pennsylvania, and North Carolina there were many fatal overdoses due to acetylfentanyl. However, acetylfentanyl was not scheduled until 2015 by the DEA.^{47,48} The DEA reported a 300% increase in fentanyl cases from 2014 to 2015. Additionally, the CDC reported a 72% increase in synthetic opioid related deaths in that same time frame.⁴³

New fentanyl derivatives continue to appear in the U.S. and Europe. Just like all other NPS, they are difficult to detect as new structures frequently appear in clinical and forensic toxicological cases. For example, during the years 2016 and 2017, over ten new fentanyl derivatives appeared in the U.S. contributing to overdoses and fatalities. Some examples include 4-methoxy-butyryl-fentanyl, o-fluoro-fentanyl, tetrahydrofuranylfentanyl, and cyclopropylfentanyl.⁴⁵ The prevalence of synthetic opioids in the U.S. is a public health

threat. It is important to be able to detect them and control them in order to combat the opioid epidemic in the U.S. and elsewhere.

Fentanyl and many of its derivatives act upon the μ -, δ -, and/or κ -opioid receptors in the human brain.⁴³ Common effects of synthetic opioid abuse include respiratory depression, miosis, and a changed mental status. There is a lack of research around the pharmacokinetics and pharmacodynamics in humans for illicit synthetic opioids. The information that does exist is derived primarily from animal models.⁴⁵ What is known is that many fentanyl derivatives have increased potency because of their lipophilic nature, ability to cross the blood brain barrier, and their high receptor affinity.⁴³ As an example, U-47700 is 7.5 times more potent in binding to the opioid receptor than morphine.⁴³ The increased potency exhibited by many synthetic opioids can lead to life-threatening respiratory and central nervous system depression. As a result of increased potency, many synthetic opioids require a higher dose of naloxone to counteract the opioid effect, which is not always known at the time of an overdose.

2.3 Identification of Drugs of Abuse and Novel Psychoactive Substances

Biological matrices for toxicologic analysis can include but are not limited to urine, oral fluid, exhaled breath, serum, plasma, whole blood, breast milk, meconium, and hair. The matrix chosen for analysis depends on the type of test being performed, what the analytes of interest are, what fluids are can reasonably be collected, and the window of detection desired. For example, breast milk and meconium are tested when a new mother is suspected to be abusing illegal drugs and there is a possibility that the baby was exposed *in utero*.⁴⁹ Hair can be used to determine long term abuse since it is possible that the analyte of interest stays in the hair as it grows. Segmental analysis can be performed to determine

abuse during a certain time frame in someone's life.⁵⁰ Different matrices have different windows of detection that can range from minutes to potentially years.

Urine and blood are typically used for routine testing for drugs of abuse (*i.e.*, workplace testing, rehabilitation, child welfare tests, and drug-facilitated assault).⁵¹ Urine is often preferred over blood for routine testing for a number of reasons. Urine has a longer window of detection than blood; urine's window of detection can last days while blood is generally only a few hours. The collection process for urine is less invasive than blood and often results in a higher sample volume. A higher sample volume can be beneficial since there will be enough sample to retest if needed. Additionally, metabolites found in urine are concentrated, making them easier to detect. Even with many benefits, urine does have disadvantages when it comes to quantification.⁵² Since the metabolites are concentrated in the urine it is difficult to calculate the actual amount of parent compound in the system.⁵³ Consequently, urine is a useful matrix for screening methods, but blood is often preferred when quantitation is important. Blood has some advantages over urine for both screening and quantitation.⁵⁴ Since quantitation is easier using blood samples, screening and quantitation can be done with using just one matrix. Additionally, compared to urine, blood levels often correlate with impairment, which is generally not true for urine. Additionally, the body regulates blood volume and only allows it to vary within a small window.⁵⁴ Finally, parent drugs of abuse can often be detected in blood prior to metabolism, unlike urine where metabolites are more common.

Urine and blood are the most common sample matrices used for the detection of NPS. When urine is the matrix being analyzed it is important that metabolites are screened for, especially when synthetic cannabinoids are of interest. Typically, only metabolites of

synthetic cannabinoids are found in urine, therefore looking for parent drugs only may not be sufficient. Analysis of synthetic cannabinoid metabolites in urine can be challenging, due to their multiplicity (often up to two dozen metabolites may be present) and possible instability.^{40,42} Blood can be used as an alternative matrix to urine for NPS with longer half-lives where it is more pertinent to screen for the parent compound. Unfortunately, most NPS are not well understood or researched, therefore the metabolism and metabolites of many NPS are not known. Since many metabolites are unknown, blood may be the desired matrix since the parent ion can be screened for. It is important to consider what NPS a method is capable of screening for when choosing the ideal matrix to use.

2.3.1 Traditional Forensic Toxicological Analysis

Toxicological sample analysis for forensic and clinical laboratories typically requires two steps. The first stage is screening for a wide variety of drugs of abuse, while the second stage typically involves confirmation with a more selective and sensitive method of detection.⁵⁵ Only samples that show a positive result on the screening method move on to be confirmed via the second analysis approach. This poses a problem for the detection of NPS, since many screening methods are not designed to specifically detect them. Therefore, samples that may be positive for NPS may be overlooked and never confirmed.

Forensic toxicological analysis is well understood and established for common drugs of abuse. The Substance Abuse and Mental Health Services Administration (SAMHSA) is an agency under the Department of Health and Human Services. SAMHSA has developed the term SAMHSA 5, which are five drug group analytes that are typically tested for in clinical and forensic toxicology laboratories. These five groups of drugs include phencyclidine (PCP), cocaine, amphetamines, THC, and opiates. Forensic and clinical

laboratories have well established protocols for testing the SAMHSA 5, unfortunately those protocols are inadequate for assessing the presence of NPS that may be found in case samples.

2.3.2 Immunoassays

Immunoassays are one of the most common screening tools used in toxicology, followed by the use of GC-MS or LC-MS for confirmation. Immunoassays are an effective, inexpensive, and rapid screening method for common drugs of abuse but have their disadvantages when it comes to screening for NPS.⁵⁶ Immunoassays are designed to react with a specific structure or functional group. Since NPS are constantly undergoing structural changes, it is unlikely that they will show cross reactivity with immunoassays commonly used in forensic and clinical toxicology.

Frequently used immunoassays include enzyme linked immune sorbent assay (ELISA) and enzyme multiplied immunoassay technique (EMIT). Unfortunately, since NPS continuously undergo structural changes it is difficult to detect new compounds emerging on the market. There are disadvantages when using immunoassays to screen for NPS. Immunoassays work through an antigen-antibody interaction, which relies on the structure of the analyte in order to show cross reactivity, which is required for a positive result. Typically, each immunoassay only cross reacts with a small number of compounds, since the antigen-antibody interaction must be specific in order to be selective. When new NPS appear on the market they often are missed by immunoassay screenings since the antibody often is not capable of reacting with a structure different than originally intended.

There have been numerous research papers published that tested the cross reactivity of NPS with different immunoassays.⁵⁷⁻⁵⁹ An example is the work accomplished by

Swortwood et al. demonstrating the lack of cross reactivity between various NPS and commonly used immunoassays.⁶⁰ Beck et al. published research looking into the cross-reactivity of NPS with commercially available immunoassays. Their findings revealed that many of the NPS tested do cross react with commercial immunoassays, leading to an issue of potential false positives. They proposed that these commercial immunoassays could potentially be used to detect for the NPS that showed cross-reactivity.⁵⁹ This can pose some issues since compounds are cross-reacting with immunoassays that are not designed to detect them, making the immunoassay less selective and further needing to rely on secondary testing for confirmation.

Recently, there have been immunoassays developed specifically for the detection of NPS, but the creation of new immunoassays can be expensive and a very lengthy process.^{61,62} In 2011 Wang et al. published data on an immunoassay designed to detect fentanyl in urine.⁶³ However, as different fentanyl derivatives became a concern, the immunoassay did not show cross reactivity with the derivatives. Randox Toxicology does have some commercial ELISAs for fentanyl, MT-45, AH-7921, and U-47700, however, this is a small subset of NPS that are available. Ellefsen et al. validated a commercially available immunoassay for synthetic cathinones in urine showing that there is substantial cross-reactivity with a number of synthetic cathinones.⁶⁴ Because of the high risk of false negatives and false positives for NPS using commercial immunoassays, a reliable screening method needs to be available that can easily be adapted for the everchanging structures of NPS that can be seen in clinical and forensic cases.

2.3.3 Instrumental Analysis

Instrumental analysis tends to be more selective and sensitive than other analysis methods. The most common instrumentation used in clinical and forensic toxicology laboratories are GC-MS and LC-MS. Such instrumentation can provide high through-put and more sensitive and selective results than other screening methods. Instrumental analysis is most commonly used for confirmation and is also capable of quantitating drug compounds in biological matrices. Instrumental analysis can also be used for screening purposes.

2.3.3.1 Gas Chromatography Mass Spectrometry

Gas chromatography mass spectrometry (GC-MS) has been the gold standard in toxicological screening for many years. Gas chromatography is well understood and established in many laboratories. It is a rugged, selective, and sensitive technique that can be used for a number of applications including screening for drugs of abuse.

Gas chromatography can be coupled to different mass spectrometers for a variety of detection purposes. There are different types of sources that can be utilized for GC-MS. Ionization sources can either be hard or soft. Hard ionization sources are extremely energetic and result in extreme fragmentation. Soft ionization techniques only produce ions of the molecular species being analyzed.⁶⁵ There are three ionization sources that are typically used in GC-MS, including electron ionization, chemical ionization, and field ionization.⁶⁵ Electron ionization, chemical ionization, and field ionization are generally considered to be hard, intermediate, and soft ionization techniques, respectively.

Gas chromatography MS is excellent for the detection of volatile, non-polar, and thermally stable compounds. However, many other compounds require derivatization

before analysis. Gas chromatography MS has increased resolving ability and selectivity as compared to LC-MS. Even though there are a number of benefits associated with GC-MS, it is also has disadvantages that need to be considered when determining the appropriate analysis technique for drug compounds in biological fluids. Depending on the matrix of the sample that is being tested, GC-MS may require extensive sample preparation such as derivatization before analysis.

2.3.3.2 Liquid Chromatography Mass Spectrometry

Recently, many laboratories have moved towards using liquid chromatography mass spectrometry (LC-MS) for clinical and forensic toxicological analysis.^{66,67} There are a number of benefits of using LC-MS over GC-MS for biological samples. One benefit is the ease of sample preparation, since there is no need for derivatization. Liquid chromatography MS is a very good technique for the detection of non-volatile, polar, and thermally-labile compounds. This is important because many drugs of abuse and NPS fall into this category of analyte. These types of compounds need to be analyzed using a direct ion source. These sources can either be in liquid phase or solid state. Analytes are in a liquid state for liquid phase ionization and introduced to the source using nebulization.⁶⁵ Examples of liquid phase ionization include electrospray ionization, atmospheric pressure chemical ionization, and atmospheric pressure photoionization.

Electrospray ionization (ESI) is a very common source used with LC-MS. Originally, ESI was most commonly used for the analysis of proteins, but later it was adapted for other polymers, biopolymers, and small polar molecules.⁶⁵ Electrospray ionization is appealing

for LC-MS since it allows for high sensitivity and can easily be coupled to LC. Electrospray ionization is formed by applying a strong electric field, under atmospheric pressure, to a liquid as it passes through a capillary tube. A high potential difference is applied between the capillary and the counter electrode to acquire the electric field. The field produces a charge build up at the liquid surface at the end of the capillary, which disperses to form charged droplets creating a Taylor cone.⁶⁸ The droplets then pass through either an inert gas or a heated capillary to remove the remaining solvent. Figure 1 is a schematic of the ESI process.

Figure 1. Represents the ESI process⁶⁸

Most commonly, tandem mass spectrometers are used in forensic toxicology laboratories for screening and confirmation of drug compounds in biological matrices. Triple quadrupole mass spectrometers (QqQ-MS) are one example of a tandem mass spectrometer that is commonly used in forensic toxicology laboratories. The first quadrupole sifts out a precursor ion, the second is a collision cell for fragmentation, and the third selects the product ions for detection. Figure 2 is a schematic of a triple quadrupole mass spectrometer.

Figure 2. Schematic of a triple quadrupole mass spectrometer showing the function of each quadrupole.

There are four main scanning modes that can be used with tandem mass spectrometry. These scanning modes include product ion scan, precursor ion scan, neutral loss scan, and selected reaction monitoring (SRM). In product ion scan mode, specific precursor ions (*i.e.*, specific m/z ratio) are selected for in the first quadrupole, then they go through fragmentation, and finally the third quadrupole scans for all precursor ions resulting from the chosen product ion. In precursor ion scan mode, the precursor ions are scanned for in the first quadrupole and then after fragmentation specific product ions are targeted and looked for in the third quadrupole. In neutral loss scan mode, all precursor ions are scanned for in the first quadrupole, they undergo fragmentation and then the third quadrupole is offset by a selected neutral loss and scanned. Lastly, during SRM, the first and third quadrupole both have m/z ratios that have been targeted for. Selected reaction monitoring can either be single reaction monitoring, which targets one precursor ion and one product ion or multiple reaction monitoring (MRM) targeting multiples transitions of each precursor ion.

There are two main acquisition techniques associated with tandem mass spectrometry, targeted and non-targeted. Targeted methods can provide extremely low LOD and are ideal for quantification.⁶⁹ Targeted analysis in forensic toxicology labs is often accomplished

using an MRM method and LC-QqQ-MS.⁶⁹ Most MRM methods target two to three transitions per analyte of interest. When using MRM, analytes of interest are detected in samples through the comparison of retention times and ion ratios. Targeted MS/MS approaches typically require database searches or library matches. There are LC-MS libraries available containing a large number of forensically significant compounds. Some examples of libraries and databases are the Wiley Registry MSMS and the NIST 11 MSMS library.⁷⁰ Dresen et al. developed an ESI MS/MS library containing 800 forensically relevant compounds in 2006 and added an additional 453 compounds in 2009.^{71,72} Electrospray ionization MS/MS libraries are widely used in clinical and forensic toxicology.

There are several libraries available for both low resolution and high-resolution MS instruments. Compounds of interest are typically identified in clinical and forensic toxicological samples on the basis of library matches.⁶⁷ Therefore, in order to identify any analyte of interest, it must be in available libraries and/or databases. Non-targeted analysis using HRMS is possible; however, it is not well established in forensic toxicology laboratories at the present time. While non-targeted analysis may become more routine in clinical and forensic toxicology laboratories in the future, current approaches generally utilize available databases and libraries to identify NPS in case samples.

As an alternative to traditionally used low resolution (unit) mass spectrometers, high resolution mass spectrometers (HRMS) are also used in some forensic laboratories for screening purposes. HRMS has increased in popularity over the past decade because of increased selectivity over low resolution MS. There are several reviews and research articles published focusing on HRMS for clinical and forensic toxicology applications.⁷³⁻

⁷⁶ Time of flight (TOF) and orbitrap are key examples of tandem HRMS. Disadvantages of HRMS for clinical and forensic toxicological analysis include the cost of the instrumentation, complexity of data analysis software, and the need for a skilled operator.⁷⁷ Regardless, HRMS is an excellent technique for screening for drugs of abuse. However, low resolution LC-MS/MS techniques (*i.e.*, LC-QqQ-MS) are still the standard in many forensic laboratories for quantitation.⁶⁶

In 2010 Wohlfarth et al. published a paper describing a LC-MS/MS method for detecting a number of NPS in serum. The classes of NPS included in their work were synthetic amphetamines, tryptamines, and piperazines. They were able to screen for a total of 35 NPS.⁷⁸ Extensive research on screening and confirmatory methods for use in detecting NPS in different biological matrices has been done since then. Ammann et al. created methods for the detection and quantification of NPS in blood, specifically targeting synthetic cannabinoids and designer cathinones.^{79,79} Adamowicz and Tokarczyk developed a method for the rapid screening of 143 NPS by LC-MS/MS. The compounds they focused on varied widely in drug class.⁸⁰ A screening and quantitative method was designed by Glicksberg et al. to detect synthetic cathinones in urine and whole blood using LC/QTOF. Their method was designed to detect 22 NPS, which is a small subset of the NPS that have been reported.⁸¹ Swortwood et al. created a method for the LC-QqQ-MS capable of screening for 32 cathinones and tryptamines in serum.⁸²

Work has also been done focusing on the detection of synthetic cannabinoids and their metabolites in urine using LC-MS/MS.^{42,83} These are examples of class-based methods; with the number of NPS currently available and their varying classes it is important that there exists a more comprehensive method. There are a number of methods that have been

created that are capable of screening for a much higher number of NPS that are not class focused. For example, recently Patridge et al. created and validated a method for the screening of 320 compounds, including several NPS, using LC-QTOF-MS. However, Patridge's method was only designed to quantitate 39 of the 320 compounds.⁸⁴ Vaiano et al published a screening method for 64 NPS in blood using LC-MS/MS. The importance of Vaiano's research was its application to real case samples.⁸⁵ Vainano's research is a prime example of the applicability of LC-MS/MS for screening NPS in biological matrices. Work has been published suggesting that LC-MS/MS is a beneficial alternative to immunoassays for screening many drugs of abuse for forensic toxicology.⁸⁶

An increased number of NPS have been detected in clinical and forensic toxicological samples over the past two decades and extensive research has been accomplished in order to combat the detection issues that are associated with NPS. However, there are still several gaps in the research revolving around the detection of NPS. Many of the published methods are only class focused and can only detect a small subset of the possible NPS in that class, especially for synthetic cannabinoids. Even though there are more comprehensive methods available, many of them lack the ability to quantify samples. With the quantity of NPS that are available to consumers and their potential to cause overdose toxicity and death, it is important to have a comprehensive screening and confirmatory method for NPS.

2.4 Extraction Methods

2.4.1 Extraction Methods for Common Drugs of Abuse

Toxicological analysis is completed by analyzing an array of biological fluids, each requiring extraction or purification to detect the analytes of interest. The most effective

extraction technique highly depends on the matrix the sample is in and the analytes of interest. Some commonly used extraction methods include dilute-and-shoot, crash-and-shoot, liquid-liquid extraction (LLE), and solid phase extraction (SPE).⁵⁵

Matrix effects are of major concern when deciding on a proper extraction and detection methods for different drugs of abuse from biological matrices. The ionization step in LC-MS is susceptible to matrix effects.^{67,87,88} Matrix effects are caused by the presence of co-eluting compounds that can increase or decrease the signal of the analyte of interest. An increase of signal is known as ion enhancement and a decrease of signal is referred to as ion suppression. Matrix effects are not always detrimental to an LC-MS method, but can be an issue when detecting analytes of interest in the lower limits of detection and quantitation of the method or when accurate determination of concentration is important (*e.g.*, driving under the influence cases).

Dilute-and-shoot is a common method used in forensic toxicology laboratories for the analysis of urine samples, which involves diluting urine samples with water before analysis. The dilution of the urine samples is necessary to protect instrumentation from the high salt concentration that can be present in urine. The dilution aids in decreasing potential matrix effects in the urine samples although it does not remove them. Crash-and-shoot involves denaturing and precipitating out proteins from whole blood, plasma, or serum samples. This is done by adding cold solvent to the sample and then centrifuging the sample to pellet and remove cellular material and proteins. The supernatant can then be used for analysis. The addition of the solvent will remove most proteins that could damage instrumentation and/or cause matrix effects.

2.4.2 Solid Phase Extraction

Solid phase extraction (SPE) is a common extraction technique used in forensic toxicology labs. Solid phase extraction is a robust technique that is capable of removing the majority of matrix interferences from various biological matrices, including urine, blood, and hair. Solid phase extraction is composed of four main steps; conditioning, loading, washing, and eluting. Conditioning is done to wet and alter the pH of the extraction cartridge so that the analytes of interest are capable of attaching to the cartridge during the loading process. Cartridges can be made of different adsorbent materials, all of which have different uses. The loading step is completed by slowly running the sample through the cartridge. Washing is done in order to remove any unwanted compounds/substances from the cartridge before elution. Elution occurs when the analytes of interest are removed from the cartridge and recovered in a solution that can then be analyzed and which should be free of most of the contaminants originally present in the complicated matrix.

Solid phase extraction is well understood and researched for common drugs of abuse. Applications of SPE for NPS are less studied and typically are adaptations of protocols for common drugs of abuse of similar classes. Since many NPS have undergone structural alterations changing their chemical interactions, Solid phase extraction protocols may need to be altered in order to properly extract NPS from different matrices. Solid phase extraction relies heavily on the chemical structure of the analytes of interest in order to retain them on the SPE cartridge and to elute them during the proper stage.

2.4.2.1 Online Solid Phase Extraction

Online SPE is an alternative to classical SPE which is designed to decrease the time and overall cost of extracting compounds from various matrices. The extraction method is

both automated and in line with the instrumentation, which eliminates a number of transfer steps, which can result in increased recovery of the analytes of interest. Often, online SPE is used as an additional cleanup step for environmental water samples.⁸⁹ However, work has been done with online SPE for the extraction of drugs of abuse. Heuett and co-workers developed an online SPE method for the extraction of common drugs of abuse from waste water.⁹⁰ Moosavi et al. developed an automated SPE method for the extraction of thiopental from plasma.⁹¹ Many of the developed methods are not specifically designed to be implemented into forensic and clinical toxicological laboratories. There is one example of work that has been done focusing on NPS using online SPE, published by Lehman et al.⁹² Their work focused on the extraction of 74 NPS from serum using online SPE LC-MS/MS. These are examples of the usefulness of online SPE for the extraction of drugs of abuse from biological matrices.

There are a number of benefits to online SPE, but it is not without its challenges. Online SPE can be very complex when developing a new method, has potential for sample loss, and sample can be retained on cartridges, which can also lead to carry over. Online SPE is also limited to the available cartridges for the system, which are not as varied as the options for classical SPE.

2.4.3 QuEChERS

The QuEChERS (Quick, Easy, Cheap, Effective, Rugged, and Safe) extraction technique was originally developed in 2003 by Anastassiades et al. to extract pesticides from a wide variety of produce.⁹³ Since then many environmental laboratories have utilized QuEChERS for different complex matrices, including food, soil samples, and invertebrates.⁹⁴⁻⁹⁶ In general, QuEChERS is an ideal extraction method for extremely dirty

and complex samples. The QuEChERS technique was originally designed as a two-step process. The first is a drying and partitioning step, while the second step involves dispersive SPE (d-SPE).⁹³ During the first step, acetonitrile is added to the sample so that liquid-liquid partitioning can be performed by adding anhydrous magnesium sulfate and sodium chloride. Once the first step is completed, a specific volume of the acetonitrile layer is removed and added to MgSO₄ and a sorbent (typically primary secondary amines; PSA) is added to accomplish d-SPE.

Companies such as Agilent Technologies and UCT have developed commercial QuEChERS kits for extraction with various applications, typically advertised for environmental samples, which require large sample volumes. However, some manufacturers do sell QuEChERS kits compatible with small sample sizes.

The QuEChERS approach has also been employed in forensic applications using a variety of biological matrices including whole blood and liver tissue.⁹⁷⁻⁹⁹ Different research groups have tested a number of different approaches using different sample sizes, combinations of salts and sorbents, and one and two step methods. In 2013, Matsuta et al. designed a one-pot extraction method for 13 compounds of various classes and metabolites in blood. Matsuta's work was one of the earlier examples using QuEChERS for a forensic application.¹⁰⁰ Westland and Dorman also published work in 2013 revolving around using QuEChERS for biological matrices. Their work focused on extracting benzodiazepines from sheep blood and human urine.⁹⁹ Soon after, Usui et al. published their QuEChERS method for extracting drugs of abuse from liver samples. Their method was applied to forensic toxicological case samples, showing the potential of QuEChERS for case work.⁹⁷ Anzillotti et al. designed a cleanup up method for drugs of abuse and benzodiazepines

using QuEChERS, showing further applications of QuEChERS in the field of forensic toxicology.¹⁰¹ Dulaurent et al. designed a QuEChERS approach for a broader set of drug classes including opiates, amphetamines and cocaine in whole blood.¹⁰² Recently Dybowski and Dawidowicz published a QuEChERS method for Δ^9 -tetrahydrocannabinol and its metabolites in whole blood.¹⁰³ Pouliopoulos et al. designed a QuEChERS approach for the detection of psychotropic drugs in postmortem blood samples.¹⁰⁴

The publications described above show the potential of QuEChERS in the field of forensic toxicology for the extraction of drugs of abuse from various biological matrices. There are advantages and disadvantages to this approach as described in current literature. Some of these methods require a large volume of sample, which is not ideal for case work. A mini one-pot approach is ideal for forensic applications. Case work samples usually have a limited volume to work with and require high throughput. Using a mini one-pot approach limits the amount of sample needed, cuts down on time and transfer steps, which makes it an ideal alternative extraction technique for forensic toxicology samples.

2.4.4 Evaluation of Extraction Techniques

All of the extraction techniques discussed need to be evaluated and optimized for NPS and compared to determine the benefits and costs associated with each method. There is published literature on the use of SPE for the extraction of NPS from blood and serum.^{75,78,82} The majority of methods published for the detection of NPS in serum or blood utilized SPE or a form of protein precipitation.^{42,80,82,105} There is very little published data solely focusing on the extraction of NPS from biological fluids, the focus is typically on the detection method. Little research using online SPE and QuEChERS has been reported for the extraction of NPS from biological fluids. Lehmann et al. published a method

capable of detecting 74 NPS using in-line SPE LC-MS/MS. Lehmann's research is one of the few examples of the use of automated SPE for the extraction of NPS.⁹² It is important that the usefulness of these techniques be tested for the extraction of NPS so that they can be implemented into forensic toxicology laboratories. It is not always possible to use a method designed for common drugs of abuse for NPS, often times they need to be optimized specifically for the extraction of NPS. Optimized extraction techniques for NPS resulting in increased recovery of NPS from biological matrices can be beneficial for forensic toxicological laboratories.

3. DEVELOPMENT OF A DYNAMIC MULTIPLE REACTION MONITORING METHOD

3.1 Introduction

Novel psychoactive substances are a global health hazard. Novel psychoactive substances are structural alterations of drugs of abuse that are manufactured in order to evade drug laws.² The detection of NPS poses difficulties for clinical and forensic toxicological laboratories because of the structural alterations. Immunoassays are commonly used for screening biological matrices. However, immunoassays are not capable of detecting the majority of NPS.^{82,86} Immunoassays are designed to detect specific drug structures or classes of drug compounds. Therefore, immunoassays are not appropriate for detecting NPS unless specifically designed to do so. There are a few immunoassays capable of screening for a small number of NPS, which still leaves out a large number of NPS that can be found in clinical and forensic toxicological samples.^{61,64} As an alternative to immunoassays, MS-based analytical techniques can be used to screen biological matrices for drugs of abuse. Mass spectrometry-based techniques (*e.g.*, GC-MS and LC-MS) typically require a spectral library or compound database in order to screen for compounds. There are a limited number of LC-MS libraries and databases that include NPS. In order to detect NPS they must be included in the spectral libraries and databases for positive matches.

The research presented here reports the collection of MS transitions and development of a comprehensive dMRM method for the detection of 750 chemical compounds, the majority of which can be considered NPS and metabolites. Transitions were collected for all 750 compounds and 76 additional deuterated internal standard compounds. Of the total

number of compounds, a final method was developed to detect 731 NPS and 22 internal standards, with two transitions per compound.

3.2 Method and Materials

3.2.1 Chemicals and Materials

Reference standards for the NPS compounds, including deuterated standards, were obtained from Cayman Chemical (Ann Arbor, MI) as the neat solid material for the majority of compounds, although some were already in solution. Optima LCMS grade methanol (MeOH), acetonitrile, dichloromethane, dimethyl sulfoxide (DMSO), HPLC water, ammonium formate (99%), and formic acid were purchased from Fisher Scientific (Fair Lawn, NJ).

3.2.2 Standards and Sample Preparation

Neat standards (including deuterated compounds) were dissolved in MeOH or DMSO, depending on the analyte's solubility, to achieve concentrations of 1, 2, 5, or 10 mg/mL. From these initial preparations, 10 µg/mL working solutions were prepared in MeOH for all analytes to be used for transition optimization and method development. In addition, from the 10 µg/mL solutions, 1 µg/mL solutions were prepared in methanol for each of the analytes to collect transitions and LC retention times in order to create the final dMRM method. An arginine reference standard from Cayman Chemical was used as a quality control standard and run daily.

3.2.3 Instrumentation and Software

All samples were analyzed using an Agilent 1290 Infinity Binary Pump LC coupled to an Agilent 6460 triple quadrupole MS/MS with Jet Streaming technology and electrospray ionization (ESI) equipped with Agilent MassHunter software version B7.0.

Chromatographic separation was performed using an Agilent Zorbax Rapid Resolution HD Eclipse Plus C₁₈ column (3.0 x 100 mm, 1.8 µm). Data acquisition was performed in dMRM mode using positive ESI. The dMRM method employed two transitions for each analyte and internal standard, which aids in achieving increased selectivity. Using multiple transitions can help in discerning one compound from another if they have similar retention times or coelute, provided that they have uniquely different transitions.

3.2.4 Methods

All standards were initially analyzed by flow injection analysis (FIA; without LC column) by QqQ-MS using the 1 µg/mL working dilution. Diluted standards were individually injected directly into the Jet Stream ESI ion source. Data were collected in positive ion mode using an isocratic mobile phase of 80:20 0.1% formic acid in methanol:5 mM ammonium formate with 0.1% formic acid in HPLC grade water. If FIA was successful, the standards were then analyzed using Optimizer software, which searches for 4 to 10 product ion transitions that are analyzed via an Optimizer Report. The report includes precursor ion, fragmentor voltage, product ions identified, collision energies, and abundances. All compounds that had four or more transitions with ion abundances above 1000 counts were then separated by LC to obtain standardized retention times.

Collected transitions were used to develop a dMRM method. Chromatographic separation was achieved using gradient elution with a flow rate of 0.3 mL/min using 5 mM ammonium formate/0.1% formic acid in HPLC water as mobile phase A and MeOH with 0.1% formic acid as mobile phase B. The gradient used for analysis was as follows: hold at 5% B for 1 min, followed by 5% B to 98% B from 1 to 9.5 min, then hold at 98% B until

16 min, followed by a 3-min re-equilibration at 5% B. The analytical column was held at a temperature of 40°C during separation.

The MS source parameters were as follows: gas temperature, 325°C; gas flow 6 L/min; nebulizer 40 psi; sheath gas temperature 350°C; sheath gas flow 11 L/min; capillary voltage 4,000 V; and nozzle voltage 750 V. Agilent MassHunter Optimizer software was used to determine the ideal data acquisition parameters for MRM mode. A dMRM method was chosen to increase selectivity, using analyte retention times, detection windows (Δt_R), and constant scan cycle time to allow for the detection of multiple analytes in a small window. Analyte detection windows ranged from 0.25 min (*i.e.*, ± 0.125 min around t_R) to 0.75 min (*i.e.*, ± 0.375 min around t_R) depending on the analyte.

To collect retention times for method development, individual compounds were injected at concentrations of 1 $\mu\text{g/mL}$ in MeOH at volumes of 3 μL . Separation was conducted over 16 min using an Agilent Zorbax Eclipse Plus C₁₈ Rapid Resolution HD column (3.0 x 100 mm; 1.8 μm) and the LC-QqQ-MS method described above. Retention time data were collected using a dMRM method with all retention times set to 8 min and a window of 16 min so that there was a continuous scan. The “Find by MRM” function of MassHunter Qualitative Analysis software was used to isolate the individual compound from each injected solution and the corresponding retention data were recorded.

3.3 Results and Discussion

Agilent MassHunter Optimizer software was used in order to identify the up to 10 fragments, associated collision energy, and optimal fragmentor voltage for each of the analytes included in this method. Appendix 1 shows the in-house identifier, compound name, chemical formula, precursor ion, transitions, collision energies, and abundances for

all compounds that underwent optimization and were included in the final method. From these data the two most abundant and/or most individualized transitions were chosen for each compound and included in the final dMRM method.

Not all of the compounds analyzed by FIA showed the expected m/z ratio in positive mode. Since positive mode was found to be appropriate for the majority of NPS to be included in this method, any compound that required negative mode ionization was excluded. The majority of compounds that required negative mode ionization were synthetic cannabinoids, with a few exceptions. Some of the excluded compounds include delorazepam, THJ 018, multiple CP cannabinoids, and a few RCS cannabinoids.

The information described above was then used to create a dMRM method capable of qualifying and quantifying the analytes of interest that require positive mode ionization. The LC gradient was chosen to separate as many compounds as possible during a 16-min run. The use of a dMRM method allowed for increased selectivity and for compounds with similar retention times but different transitions to be differentiated. An example would be 25I-NBMD and bromazepam, both with $t_R = 9.00$ min, which could be separately identified as a result of their unique transitions.

The final dMRM method included two transitions each for 750 compounds and 22 deuterated internal standards. Table 1 depicts the breakdown of drug entities included in the final method based on drug class. Table 2 depicts the distribution of compounds in the final method based on molecule type. The goal of this dMRM method was to be as comprehensive as possible, based on available standards, for the detection of NPS in clinical and forensic toxicological samples. Common adulterants found with illicit drug

samples are one of the sub-categories that fall under the “other” category. The method is designed to detect NPS and their common adulterants in case samples.

Table 1. Structural classes for all compounds included in the dMRM database.

Drug Class	Number in Method
Synthetic Cannabinoids	449
Other*	121
Cathinone	112
Phenethylamine	43
Tryptamine	17
Piperazine	8

* includes opioids, amphetamines, benzofuran, and common adulterants

Table 2. Molecule types for all compounds included in the dMRM database.

Molecule Type	Number in Method
Precursor Compounds	470
Metabolites	117
Isomers	128
Analogs	30
Glucuronides	5

The final MRM database included data for 826 individual analytes including 76 deuterated standards (see Appendix). However, the final dMRM method was unable to include transitions for all of the compounds in the database in a single MS run. This limitation is caused by the instrument’s ability to collect usable data, which relies greatly on cycle time and dwell time. Dwell time is the amount of time in ms that it takes to collect one transition, while cycle time is the time it takes in ms to collect all transitions associated with a compound.

$$dwell\ time\ (ms) = \frac{peak\ width\ (ms)}{(number\ of\ transitions)(number\ of\ points)} \quad (1)$$

Dwell time is determined using equation 1. The issue with having too many transitions (*i.e.*, >800) is that there will not be enough points on the peaks of the data collected in order to quantitate the peak area. Ideally there should be about 20 points on the peak; with 1000+ transitions it is impossible to have enough points while still maintaining a reasonable dwell time. In a dMRM method there is a list of transitions that the instrument needs to scan through. Inclusion of all 826 compounds, with two transitions each, would require 1652 transitions. A specific retention time and window is assigned to every compound. Throughout this window the instrument needs to go through the scan 20 times in order to have 20 points on the peak. For certain compounds, retention time windows overlap, therefore they are sharing the total cycle time and that will change the dwell time for each transition. If the dwell time becomes too low the data will not be reproducible or statistically relevant. There are only so many transitions that can share the same retention window. If there are too many sharing the same cycle time, when the instrument tries to cycle through the transitions 20 times it will be trying to collect data from a peak that has already been eluted, because it cannot cycle through fast enough.

Additionally, when attempting to collect such a high number of peaks in a single 16 min run, the resolution between peaks would be very low. Consequently, since there are limitations associated with dwell time and cycle time, it is not possible to measure 800+ compounds in a single dMRM method. Therefore, in order to use the method described, it is necessary to break it into two separate screening runs. This is needed so that the quality of the resulting data are not compromised. For forensic toxicological laboratories to implement this method, each sample would therefore need to be run twice, resulting in a

32-min rather than 16 min run. This should be acceptable considering the advantages of screening for so many NPS in each specimen.

3.4 Conclusions

The work presented here aimed to create a dMRM method capable of screening for 750 NPS. After undergoing flow injection analysis, it was determined that 729 of the total number of NPS were suitable for positive ion mode. Those 729 NPS were included in the final developed dMRM. The final method is intended to screen for a variety of NPS including metabolites. In order to use this method for forensic purposes it needs to be fully validated.

4. METHOD VALIDATION USING A MIXTURE APPROACH

4.1 Introduction

In recent years, “designer drugs,” also known as novel psychoactive substances (NPS), have become of major concern all over the world, especially in the United States. Novel psychoactive substances are compounds that are considered to be “substantially similar” to Schedule I or II substances determined by chemical structure and pharmacological effects, but that have not been scheduled and therefore are not yet “illegal”.¹⁰⁶ Suppliers and consumers use NPS to evade established drug laws. Since every small structural change can result in a new NPS, these compounds are constantly increasing in numbers, making it very difficult for clinical and forensic laboratories to keep up with detection and identification.¹⁰⁷ The popularity of NPS continues to increase, as reflected by Internet content, the media, published scientific research, and the types of forensic and clinical cases reported, including reported fatalities and unexpected side effects.^{108,109} It is not uncommon for NPS to have a more potent effect than their scheduled counterparts, leading to more cases of overdose and increased negative side effects. A recent example of potent NPS are the fentanyl derivatives that have been seen during the recent opioid crisis in the United States.⁴³⁻⁴⁵ The continuous rise of NPS makes it clear that new detection methods are needed in order to keep up with the changing structures of compounds being abused.

With the increased importance of reliable detection of NPS in forensic casework, research focusing on creating and validating methods capable of detecting NPS has accelerated.^{77,82,110} The majority of published methods that focus on detecting NPS fall into two categories; those that provide quantitation of a relatively small number of NPS and those that can screen for (but not quantitate) a larger number of NPS. As a consequence of

the limitations of current screening/confirmatory approaches, methods need to be created that encompass both of the goals discussed above.

There are established guidelines by OSAC that need to be followed so that a toxicological method can be considered validated. There are strict instructions on what parameters need to be validated depending on the overall purpose of the method being validated (*i.e.*, qualitative or quantitative).¹¹¹ Peters et al. published additional guidelines on method validation as it refers to forensic toxicological analyses. Forensic toxicology methods must be painstakingly validated and periodically tested to ensure the quality of the results.¹¹² The main parameters for validation are LOD, LOQ, selectivity, linearity, carry over, bias, precision, freeze/thaw stability, and matrix effects. There are specific ranges of acceptable values that all of these results need to fall into in order to be considered validated. Each compound in a method must be validated for all of the parameters listed. For any method consisting of a small set of compounds it is possible to validate the method one compound at a time, however, it is more efficient to validate methods using a mixture approach.

The present work focuses on the development of a validated LC-QqQ-MS method that is designed to confirm and quantitate 800+ NPS. To fully validate a method of this size, a mixture approach was adopted. Specifically, 826 individual NPS and metabolites were incorporated into 16 non-coeluting mixtures for method validation. The mixture approach was utilized to facilitate timely method validation for such a large number of analytes, since validation of one compound at a time was not feasible. Mixture approaches have previously been used for the validation of screening methods.^{78,84} However, they are less commonly

used to validate quantitative methods. Typically, only one mixture is used for methods quantitating 40 or less compounds in total.^{80,82,113} The current work employs the mixture approach on a much wider scale, to fully validate a quantitative method capable of detecting a very large number of NPS. The present report focuses on a subset of three mixtures that have been fully validated as a proof-of-concept of this approach.

4.2 Materials and Methods

4.2.1 Chemicals and materials

Reference standards for the NPS compounds, including deuterated standards, were obtained from Cayman Chemical (Ann Arbor, MI) as the neat solid material for the majority of compounds, although some were already in solution. Abbreviations and number association that will be used for a subset of the NPS included in this work can be seen in Table 3, which also separates the compounds into their mixtures. A total of 16 mixtures were designed for method validation, the results of three of those mixtures will be shown here. All other mixtures that have successfully undergone validation can be seen in the Appendix.

Table 3. List of the abbreviations used for the NPS contained in the three mixes used for validation.

#	Mixture 1	#	Mixture 2	#	Mixture 3
1	4-OH MET	26	3,4-DHMA	58	N,N-DMC
2	NMT	27	2-FMC	59	Phenylpiperazine
3	4'-fluoro- α -PPP	28	4-FIC	60	4-hydroxy DiPT
4	4-APDB	29	4-OH MiPT	61	THH
5	4-fluoro PBP	30	Clencyclohexeral	62	MMAI
6	5-MAPB	31	NEB	63	2,3-pentylone isomer
7	3-methyl BP	32	4-MMC	64	(-)-3,4-MDPV
8	4-methyl- α -EAB	33	3-methyl PPP	65	3C-B-fly
9	α -PVP metabolite 1	34	3,4-dimethoxy- α -PVP	66	Para-Fluorofentanyl
10	4-MeO- α -PVP	35	2,3-MDPV	67	PCEEA
11	JWH 200 5-hydroxyindole metabolite	36	4-ethyl-N,N-DMC	68	Benzydamine
12	PCMPA	37	2C-T-2	69	Bromazepam

13	AM2233 azepane isomer	38	PCPr	70	AB-PINACA N-(5-hydroxypentyl) metabolite
14	4-MeO PV8	39	2C-T-4	71	25E-NBOMe
15	Benocyclidine	40	4'-Methylhexedrone	72	Etaqualone
16	25I-NBMD	41	25I-NBF	73	JWH 198
17	AB-005	42	Loperamide	74	AB-FUBINACA
18	Flubromazepam	43	AB-005 azepine isomer	75	(R)-(-)-JWH 018 N-(4-hydroxypentyl) metabolite
19	AM694 N-(5-hydroxypentyl) metabolite	44	A-796260	76	(+)-WIN 55,212-2
20	5-fluoro SDB-006	45	AB-FUBINACA 3-FB isomer	77	JWH 073 6-MeO indole analog
21	JWH 081 N-(5-hydroxypentyl) metabolite	46	JWH 018 N-(5-hydroxypentyl) metabolite	78	JWH 073 2'-naphthyl-N-(1,1-DME) isomer
22	PB-22 6-hydroxyisoquinoline isomer	47	MAM2201 N-pentanoic acid metabolite	79	BB-22 8-hydroxyisoquinoline isomer
23	NPB-22	48	ADB-PINACA isomer 1	80	JWH 019
24	JWH 203	49	RCS-4 2-MeO isomer		
25	THCA-A	50	PB-22		
		51	XLR11 N-(2-FP) isomer		
		52	UR-144 Degradant		
		53	AKB48 N-(5-FP) analog		
		54	KM 233		
		55	Δ 8-THC		
		56	EG-018		
		57	SER-601		

Optima LCMS grade methanol, acetonitrile, HPLC water, ammonium formate (99%) and formic acid were purchased from Fisher Scientific (Fair Lawn, NJ). Certified blank urine was purchased from UTAK Laboratories Inc. (Valencia, CA) and aliquoted into 10 mL portions and stored at -20°C until needed.

4.2.2 Solution and sample preparation

Neat standards (including deuterated compounds) were dissolved in methanol (MeOH) or dimethyl sulfoxide (DMSO), depending on the analyte's solubility, to achieve concentrations of 1, 2, 5, or 10 mg/mL. From these initial preparations, 10 µg/mL working

solutions were prepared in MeOH for all analytes to be used for optimization and method validation.

4.2.3 Instrumentation

All samples were analyzed using an Agilent 1290 Infinity Binary Pump LC coupled to an Agilent 6460 triple quadrupole MS/MS with Jet Streaming technology and electrospray ionization (ESI) equipped with Agilent MassHunter software version B7.0. Chromatographic separation was performed using an Agilent Zorbax Rapid Resolution HD Eclipse Plus C₁₈ column (3.0 x 100 mm, 1.8 µm). Data acquisition was performed in dMRM mode using positive ESI. The dMRM method employed two transitions for each analyte and internal standard, which aids in achieving increased selectivity. Using multiple transitions can help in discerning one compound from another if they have similar retention times/coelute, as long as they exhibit uniquely different transitions.

4.2.4 Preparation of standard mixtures

The 10 µg/mL solutions were used to prepare NPS mixtures for validation by spiking MeOH with each compound for a final concentration of 200 ng/mL per compound. The spiked MeOH solutions were used as the working solutions to create all samples needed for method validation. Validation mixtures contained anywhere from 22 to 65 compounds of varying NPS structural and pharmacological classes. The 750 compounds included in the final dMRM method were divided into a total of 16 different mixtures for ease of data analysis. Using a series of non-coeluting standard mixtures helped ensured selectivity during method validation. Compounds chosen for each mixture were determined by retention time and primary MRM transitions. Each mix included only non-coeluting compounds to ensure selectivity. In addition, an internal standard (IS) mixture of 22 compounds, each at a concentration of 200 ng/mL, was prepared to be used for

quantification. The 22 deuterated IS compounds, along with LC retention time and drug class can be found in Table 4. Internal standards were chosen in order to cover all of the drug classes included in the in the final dMRM method. As it was impossible to find a deuterated compound for every NPS used in this research, a set of internal standards were chosen in order to match structures and drug classes as well as possible for each analyte.

Table 4. List of internal standards used for validation, drug class, and retention times.

Compound	RT	Drug Class
JWH 007-d9	11.63	Synthetic Cannabinoid
JWH 018-d9	11.52	Synthetic Cannabinoid
JWH 073 5-Hydroxyindole metabolite-d7	10.69	Synthetic Cannabinoid
JWH 081 N-pentanoic acid metabolite-d5	10.54	Synthetic Cannabinoid
(-)-11-nor-9-carboxy- Δ^9 -THC-d3	11.47	Cannabinoid
($\pm$)-CP 47,497-C8-homolog-d7	12.05	Synthetic Cannabinoid
AM 2201 N-(4-hydroxypentyl) metabolite-d5	10.26	Synthetic Cannabinoid
MAM 2201 N-pentanoic acid metabolite-d5	10.67	Synthetic Cannabinoid
PB-22-d9	11.3	Synthetic Cannabinoid
UR-144 N-(4-hydroxypentyl) metabolite-d5	10.85	Synthetic Cannabinoid
XLR 11 N-(4-hydroxypentyl) metabolite-d5	11.59	Synthetic Cannabinoid
RCS-4 N-(5-hydroxypentyl) metabolite-d5	10.08	Synthetic Cannabinoid
25I-NBOMe-d3	9.18	Phenethylamine
Benocyclidine-d10	9.08	Arylcyclohexylamine
3,4-Methylenedioxy pyrovalerone-d8	7.48	Stimulant
AB-PINACA-d9	10.74	Synthetic Cannabinoid
ADB-PINACA-d9	11.03	Synthetic Cannabinoid
AB-FUBINACA-d4	10.25	Synthetic Cannabinoid
Acetyl norfentanyl-d5	5.98	Opioid
Norsufentanil-d3	7.49	Synthetic Opioid
Butylone-d3	6.53	Stimulant
cis-Tramadol-d6	7.15	Opioid

Calibrators and quality control (QC) samples were prepared using pooled certified blank urine. Sample preparation before analysis consisted of using a dilute-and-shoot method with a ratio of 1:5 (urine:HPLC water). Samples were prepared by spiking urine

with one of the NPS 200 ng/mL mixtures described above in addition to the IS spiking solution. Once spiked, the urine samples were diluted using HPLC water before undergoing LC-MS analysis.

4.2.5 LC Conditions and MS parameters

Chromatographic separation was achieved using gradient elution with a flow rate of 0.3 mL/min using 5 mM ammonium formate/0.1% formic acid in HPLC water as mobile phase A and MeOH with 0.1% formic acid as mobile phase B. The gradient used for analysis was as follows: hold at 5% B for 1 min, followed by 5% B to 98% B from 1 to 9.5 min, then hold at 98% B until 16 min, followed by a 3-min re-equilibration at 5% B. The analytical column was held at a temperature of 40°C during separation.

The MS source parameters were as follows: gas temperature, 325°C; gas flow 6 L/min; nebulizer 40 psi; sheath gas temperature 350°C; sheath gas flow 11 L/min; capillary voltage 4,000 V; nozzle voltage was 750 V. Agilent MassHunter Optimizer software was used to determine the ideal data acquisition parameters for MRM mode. The software uses the mass of each compound to determine optimal fragmentor voltage, resulting product ions, and associated collision energies and can optimize for up to 10 transitions for each precursor ion.

Prior to validation of the method, retention time data for all compounds were collected. Separation was conducted over 16 min using an Agilent Zorbax Eclipse Plus C₁₈ Rapid Resolution HD column (3.0 x 100 mm; 1.8 µm) and the LC-QqQ-MS method described above. The “Find by MRM” function of Qualitative Analysis software was used to isolate the individual compound from each injected solution and the corresponding retention data were recorded. These data were used to design the validation mixtures so that no two

components of a mixture would co-elute, thus minimizing interference with identification and quantitation of the compounds.

4.2.6 Quantification

Agilent MassHunter Quantitative Analysis software version B.07.00 was used for quantification. The software was used to calculate and plot peak area ratios of drug versus internal standard. Using the calibration curves produced, the software then calculated the concentration of each sample.

4.2.7 Assay validation

The LC-MS/MS method was validated in accordance with guidelines for forensic toxicology method validation provided by OSAC and as described by Peters et al.¹⁰⁷⁻¹⁰⁸ The parameters evaluated consisted of selectivity, matrix effects, recovery, linearity, freeze-thaw stability, carry over, accuracy, and precision.

4.2.8 Selectivity

Blank pooled urine samples were prepared using the dilute-and-shoot procedure described above and analyzed using the dMRM method to ensure that there were no peaks present that could interfere with the analytes of interest or internal standards. Blank urine was spiked with the IS mixture at a concentration of 100 ng/mL and analyzed to confirm that the internal standard peaks did not interfere with the detection of the targeted analytes. Lastly, each of the NPS mixtures were spiked into urine and analyzed to ensure that the targeted analytes did not interfere with the internal standard peaks.

4.2.9 Matrix effects and recovery

Traditionally, matrix effects are determined by comparing three different samples sets

(*i.e.*, analyte of interest in solvent, analyte of interest spiked after extraction, and analyte of interest spiked before extraction); the described approach is not feasible when using a dilute-and-shoot method. As an alternative, matrix effects were determined by comparing the results of the analytes of interest spiked in HPLC water (Set 1) and spiked into pooled urine that was diluted before analysis (Set 2). Matrix effects were determined at three different concentrations, 5 ng/mL (LOW), 20 ng/mL (MED) and 80 ng/mL (HIGH) through the comparison of peak areas. Matrix effects were calculated by using equation 2 shown below. Positive values represent ion enhancement and negative values represent ion suppression; the higher the value, the higher the level of interference. According to OSAC guidelines, a value of $\pm 25\%$ for matrix effect is acceptable for method validation.

$$Matrix\ Effect = \left(\frac{Set\ 1 - Set\ 2}{Set\ 1} \right) * 100 \quad (2)$$

4.2.10 Linearity of calibration and limits of detection/quantitation

Calibration curves were analyzed by using seven calibration levels ranging from 1 to 100 ng/mL (*i.e.*, 1, 2, 5, 10, 20, 50, and 100 ng/mL). Each of the 22 IS in the IS spiking mixture were present in each calibrator at a concentration of 40 ng/mL. Each calibration level was prepared in pooled blank urine at a volume of 0.4 mL. Replicates (n=4) at each concentration were analyzed using the dMRM method described above over the course of five different days. Regression lines were calculated for each analyte of interest using Agilent MassHunter Quantitative Analysis software with a weighted (1/x) model.

Limit of detection and LOQ were determined using Equations 3 and 4, respectively. Using the calibration curve to derive LOD and LOQ was deemed a viable option since all calibration curves were linear. Alternative methods for determining LOD and LOQ could have been employed, but this approach was appealing since it did not include additional

analysis, which can shorten the time required for method validation when working with a large number of compounds. The described approach uses the equation of the line, where s_y is the standard deviation of the y-intercept and Avg_m is the average slope of the line:

$$LOD = (3.3s_y)/Avg_m \quad (3)$$

$$LOQ = (10s_y)/Avg_m \quad (4)$$

4.2.11 Precision and accuracy

Precision and accuracy were determined through the analysis of QC samples at three different concentrations, 5, 20, and 80 ng/mL. Each QC concentration level was analyzed in replicates (n=3) over five different days. The mean value for each concentration on each day were used to determine interday bias and precision. Each replicate was used to determine intraday bias and precision. Equations 5 and 6 are used to determine interday and intraday percent coefficient of variance (%CV) values, respectively. According to OSAC guidelines, 20% CV and $\pm 20\%$ bias are acceptable for method validation.

$$Interday\ CV\ (\%) = \frac{std\ dev.of\ all\ observations\ for\ each\ concentration}{grand\ mean\ for\ each\ concentration} \times 100 \quad (5)$$

$$Intraday\ CV\ (\%) = \frac{std\ dev.of\ a\ single\ run\ of\ samples}{mean\ calculated\ value\ of\ a\ single\ run\ of\ samples} \times 100 \quad (6)$$

Recovery was determined using the same QC samples that were used for bias and precision studies. The average of each concentration (LOW, MED, and HIGH) with repeats (n=3) over five different runs were used to determine percent recovery. The average

concentration was divided by the expected concentration and multiplied by 100% in order to determine percent recovery.

4.2.12 Freeze-thaw stability

Freeze-thaw stability was completed over three freeze thaw cycles at two different concentrations (LOW and HIGH). At the start of the experiment, 5 mL of 5 ng/mL (LOW) and 80 ng/mL (HIGH) of the NPS mix being tested was prepared in matrix and aliquoted into four amber vials. The first vial was used for time zero and the other three vials were placed in the freezer (-20°C) for 24 h, after which they were all removed and allowed to thaw to room temperature for 2 h. After this time, one vial was analyzed as first thaw and the other two vials were placed back in the freezer for 20 h, before being thawed to room temperature for 2 h. After being thawed, one vial was analyzed as the second thaw cycle and the other was placed back into the freezer for another 20 h and then analyzed once it returned to room temperature as the third thaw. Calibration curves were made fresh daily for quantification. Mean concentrations of first, second, and third thaw samples were compared to the mean concentrations of the analytes of interest at time zero. Compounds were considered stable as long as the mean concentration stayed within $\pm 15\%$ of the mean concentration calculated at time zero.

4.3 Results

The final dMRM method that underwent method validation included two transitions each for 750 compounds and 22 deuterated internal standards. Tables 5, 6, and 7 show the dMRM parameters used for Mixes 1, 2, and 3, respectively and the internal standard match for each compound. Information regarding the dMRM parameters for all other compounds included in the final method can be seen in the Appendix.

Table 5. Dynamic MRM MS method parameters for NPS in Mix 1 and internal standard matches.

Drug	Precursor Ion	Transitions	CE (V)	Fragmentor (V)	t _R (min)	Internal Standard
4-OH MET	219.1	160 72	16 12	96	5.53	Butylone-d3
NMT	175.1	144 117	8 28	84	6.06	Benocyclidine-d10
4'-fluoro- α -PPP	222.1	123 98	24 28	120	6.31	Butylone-d3
4-APDB	178.1	161 133	8 20	84	6.56	Benocyclidine-d10
4'-fluoro- α -PPP	222.1	123 98	24 28	120	6.31	Butylone-d3
4-APDB	178.1	161 133	8 20	84	6.56	Benocyclidine-d10
4-fluoro PBP	236.1	112 109	24 28	120	6.87	Butylone-d3
5-MAPB	190.1	159 131	8 20	84	7.04	3,4-Methylenedioxy Pyrovalerone-d8
3-methyl BP	192.1	174 144	8 36	96	7.28	Benocyclidine-d10
4-methyl- α -EAB	206.2	188 144	8 32	108	7.48	Butylone-d3
α -PVP metabolite 1	234.2	216 72	16 20	108	7.62	JWH 081 N-pentanoic acid metabolite-d5
4-MeO- α -PVP	262.2	126 121	24 24	120	7.69	Butylone-d3
JWH 200 5-hydroxyindole metabolite	401.2	155 114	20 32	108	8.03	PB-22-d9
PCMPA	248.2	159 91	12 40	84	8.16	Benocyclidine-d10
AM2233 azepane isomer	459.2	58 112	60 24	108	8.55	RCS-4 N-(5- hydroxypentyl) metabolite-d5
4-MeO PV8	290.2	154	28	108	8.79	Butylone-d3

		121	24			
Benocyclidine	300.2	147 86	32 4	72	8.98	JWH 073 5-hydroxyindole metabolite-d7
25I-NBMD	442.1	135 77	32 60	120	9.00	25I-NBOMe-d3
AB-005	353.3	112 98	24 36	120	9.39	Butylone-d3
Flubromazepam	333.0	226 184	32 32	120	9.77	JWH 018-d9
AM694 N-(5-hydroxypentyl) metabolite	434.1	230.9 202.9	20 56	120	9.99	(-)-11-nor-9-carboxy- Δ 9-THC-d3
5-fluoro SDB-006	339.2	232.1 91.1	20 56	120	10.38	3,4-Methylenedioxy Pyrovalerone-d8
PB-22 6-hydroxyisoquinoline isomer	388.2	185.1 157.1	20 48	108	10.57	PB-22-d9
NPB-22	359.2	214.1 144	16 44	108	10.77	($\pm$)-CP 47,497-C8-homolog-d7
THCA-A	359.2	341.2 219.1	12 36	108	13.45	Benocyclidine-d10

Table 6. Dynamic MRM MS method parameters for NPS in Mix 2 and internal standard matches.

Drug	Precursor Ion	Transitions	CE (V)	Fragmentor (V)	t _R (min)	Internal Standard
Δ8-THC	315.2	193.2 123.1	24 36	108	12.33	(-)-11-nor-9-carboxy-Δ9-THC-d3
2,3-methylenedioxy pyrovalerone	276.2	175.0 126.1	20 32	120	7.41	3,4-Methylenedioxy Pyrovalerone-d8
25I-NBF	416.1	291.0 109.1	20 56	120	8.88	25I-NBOMe-d3
2C-T-2	242.1	225.1 91.1	8 52	72	7.88	25I-NBOMe-d3
2C-T-4	256.1	239.1 197.1	8 16	84	8.36	25I-NBOMe-d3
2-fluoromethcathinone	182.1	164.1 149.0	12 20	96	5.38	Butylone-d3
3,4-DHMA	182.1	123.0 77.1	20 44	84	4.19	Butylone-d3
3,4-dimethoxy-α-Pyrrolidinopentiophenone	292.2	151.1 126.0	28 24	120	7.15	Butylone-d3
3-methyl-α-Pyrrolidinopropiophenone	218.2	119.1 98.1	24 28	120	6.93	Butylone-d3
4'-Methyl-N-methylhexanophenone	220.2	202.2 105.1	8 24	96	8.39	Butylone-d3
4-ethyl-N,N-dimethylcathinone	206.1	105.1 72.1	28 28	120	7.43	Butylone-d3
4-fluoroisocathinone	168.1	123.0 77.1	16 40	72	5.39	Butylone-d3
4-hydroxy MiPT	233.2	160.0 86.1	20 12	96	5.86	Norsufentanil-d3
4-MMC	178.1	160.1 145.1	8 20	84	6.6	Butylone-d3
A-796260	355.2	125.1 114.1	20 32	120	10.2	UR-144 N-(4-hydroxypentyl) metabolite-d5
AB-005 azepane isomer	353.3	112.0	24	120	9.47	UR-144 N-(4-

		58.1	56			hydroxypentyl) metabolite-d5
AB-FUBINACA 3-fluorobenzyl isomer	369.2	253.0 109.0	24 52	96	10.11	AB-FUBINACA-d4
ADB-PINACA isomer 1	345.2	215.1 145.0	24 48	96	10.72	ADB-PINACA-d9
AKB48 N-(5-fluoropentyl) analog	384.2	135.1 93.0	24 60	120	11.81	AB-FUBINACA-d4
Clencyclohexerol	319.1	203.0 81.1	20 32	96	6.05	UR-144 N-(4-hydroxypentyl) metabolite-d5
EG-018	392.2	155.1 127.1	24 60	120	12.78	JWH 018-d9
JWH 018 N-(5-hydroxypentyl) metabolite	358.2	155.0 127.0	20 52	120	10.37	JWH 081 N-pentanoic acid metabolite-d5
KM 233	363.2	119.1 91.1	20 60	120	12.09	(-)-11-nor-9-carboxy- Δ^9 -THC-d3
Loperamide	477.2	266.1 210.1	24 60	120	9.22	Acetyl norfentanyl-d5
MAM2201 N-pentanoic acid metabolite	386.2	169.1 141.1	24 48	120	10.55	MAM2201 N-pentanoic acid metabolite-d5
N-Ethylbuphedrone	192.1	130.1 91.1	32 32	96	6.51	Butylone-d3
PB-22	359.2	214.1 116.0	8 60	60	11.2	PB-22-d9
PCPr	218.2	91.1 60.2	28 4	60	8.19	Benocyclindine-d10
RCS-4 2-methoxy isomer	322.2	135.0 77.1	20 60	120	10.96	RCS-4 N-(5-hydroxypentyl) metabolite-d5
SER-601	435.3	284.2 135.1	28 32	120	13.28	JWH 007-d9
UR-144 Degradant	312.2	214.1 55.2	20 60	120	11.64	UR-144 N-(4-hydroxypentyl) metabolite-d5

Table 7. Dynamic MRM MS method parameters for NPS in Mix 3 and internal standard matches

Drug	Precursor Ion	Transitions	CE (V)	Fragmentor (V)	t _R (min)	Internal Standard
(-)-3,4-Methylenedioxy Pyrovalerone	276.2	135.1 126.1	28 28	120	7.29	3,4-Methylenedioxy Pyrovalerone-d8
(+)-WIN 55,212-2	427.2	155.0 127.0	24 60	120	10.86	UR-144 N-(4-hydroxypentyl) metabolite-d5
(R)-(-)-JWH 018 N-(4-hydroxypentyl) metabolite	358.2	155.1 127.1	20 56	120	10.44	JWH 081 N-pentanoic acid metabolite-d5
2,3-pentylone isomer	236.1	188.1 131.1	12 40	108	7.24	butylone-d3
25E-NBOMe	330.2	121.1 91.1	50 52	120	9.25	25I-NBOMe-d3
3C-B-fly	298.1	281 202.1	12 24	84	7.97	25I-NBOMe-d3
4-hydroxy DiPT	261.2	160.1 114.1	20 12	96	6.48	
AB-FUBINACA	369.2	324.1 109.0	12 48	96	10.15	AB-FUBINACA-d4
AB-PINACA N-(5-hydroxypentyl) metabolite	347.2	302.1 213.0	12 28	96	9.19	AB-PINACA-d9
BB-22 8-hydroxyisoquinoline isomer	385.2	240.2 144.0	20 44	120	11.78	PB-22-d9
Benzylamine	310.2	86.1 58.1	16 56	108	8.65	AB-FUBINACA-d4
Bromazepam	316.0	209.1 182.1	28 36	108	9.00	Cis-tramadol-d6
Etaqualone	265.1	146.0 77.0	28 60	120	9.95	butylone-d3
JWH 019	356.2	228.1 144.0	24 40	120	11.82	JWH 018-d9
	328.2	154.9	28	120	11.4	

JWH 073 2'-naphthyl-N-(1,1-dimethylethyl) isomer		144.2	24			JWH 073 5-hydroxyindole metabolite-d7
JWH 073 6-methoxyindole analog	358.2	230.1 174.1	24 40	120	11.29	JWH 073 5-hydroxyindole metabolite-d7
JWH 198	415.2	185.1 114.1	24 32	120	10.07	JWH 007-d9
MMAI	178.1	161.0 105.0	8 24	84	7.15	JWH 073 5-hydroxyindole metabolite-d7
N,N-dimethylcathinone	178.1	105.1 72.1	20 24	108	5.48	butylone-d3
p-Fluorofentanyl	355.2	188.2 105.1	24 48	120	8.01	acetyl norfentanyl-d5
PCEEA	248.2	159.1 65.1	8 60	84	8.26	Benocyclidine-d10
Phenylpiperazine	163.1	120.0 77.1	20 44	108	5.77	acetyl norfentanyl-d5
Tetrahydroharmine	217.1	200.0 188.0	8 8	84	6.68	Norsufentanil-d3

4.3.1 Selectivity and carryover

A set of ten diluted purchased pooled certified blank urine samples were analyzed using the developed dMRM method to ensure that there were no interfering peaks. Initially, there was a peak identified as PB-22 6-hydroxyisoquinoline isomer that showed up consistently in every sample. In order to address this issue, the transitions for this analyte were changed and the interfering peak was no longer present when blank urine was re-analyzed. When analyzing IS and NPS drug mixtures spiked in blank urine using dMRM no interfering peaks were observed, confirming the value of using a dMRM method to eliminate interfering peaks that could be present in full scan MS modes. Interferences can be detrimental to a screening and confirmatory

test and can contribute to false positive results (*i.e.*, the detection of a compound that is not actually in the sample). It is extremely important to avoid false positive results in clinical and forensic toxicology.

Carry over can be the result of compounds not fully eluting from the analytical column and can affect quantitative analysis. In order to address any carry over, five blank matrix samples were injected after analysis of the highest calibrator (100 ng/mL) to determine if there was any carry over. When analyzing the five blank urine samples there was no carryover seen, meaning that the 3-min clean up after the 16 min run was sufficient to eliminate carry over from higher concentrated samples in the next sample or blank. Carry over can also contribute to false positive results, which should be avoided.

4.3.2 Linearity of calibration and limit of detection/quantitation

Agilent MassHunter Quantitative Analysis software was used to find regression lines for each of the analytes. Additionally, the software was used to aid in the determination of precision, accuracy, LOD, and LOQ for all analytes included in this experiment. All regression models were weighted by a factor of $1/x$ to offset heteroscedasticity. All R^2 values were a minimum of 0.95 for the analytes analyzed. However, the majority of compounds had an R^2 value >0.99 . Linear range was 1 to 100 ng/mL for most NPS analyzed. There were a few compounds that did not show linearity, including THCA-A and tetrahydroharmine. Only compounds showing linearity were further analyzed for method validation. At concentrations higher than 100 ng/mL, linearity was lost for the majority of the compounds. Nevertheless, the range used is adequate for the concentration of NPS found in typical case samples, including fatalities.¹⁰⁸ If a sample

is suspected to be above the 100 ng/mL linearity cutoff, it can be diluted before analysis, which will alleviate the issue.

Limits of detection (LOD) and limits of quantitation (LOQ) were determined using the equation of the regression line for each compound, which was possible because of the linearity of the calibration curves. Limit of detections for all compounds in Mixes 1, 2, and 3 ranged from 0.01 to 0.12 ng/mL and LOQs for all the compounds analyzed ranged from 0.02 to 0.36 ng/mL. Limit of detections and LOQs for all compounds in Mixes 1, 2, and 3 can be found in Tables 8-10, respectively. These LODs and LOQs are similar to those that have been reported previously in literature for selected NPS.^{42,80,84,85} It is important to note that LOD and LOQ were determined analyzing diluted samples, therefore the detection and quantification limits represent what is possible in a diluted sample. The ability to detect and quantify NPS in the ppt range will greatly aid in the identification and quantification of some of the more potent NPS, which are often found in low concentrations in case studies.

4.3.3 Precision and accuracy

The QC samples were analyzed at 5, 20, and 80 ng/mL in triplicate on five different days. Accuracy, precision, and percent recovery were calculated for each analyte at the three different concentrations. Acceptable values were $\pm 20\%$ for bias and 20% for precision (% CV). These values for the compounds included in Mixes 1, 2, and 3 can be found in Tables 8, 9, and 10, respectively. Bias, precision, LOD, and LOQ values for additional mixes can be found in the Appendix. All compounds in Mixes 1, 2, and 3 fell within the acceptable limits for both bias and precision.

Table 8. LOD, LOQ, R2 values, and precision and bias values for all compounds in Mix 1 at three different concentration levels.

Compound Name	R ²	LOD (ng/mL)	LOQ (ng/mL)	Low (5 ppb)		Medium (20 ppb)		High (80 ppb)	
				% CV	% Bias	% CV	% Bias	% CV	% Bias
4-OH MET	0.9985	0.039	0.117	5.5	-6.3	2.6	-5.1	3.0	-1.5
NMT	0.9982	0.040	0.121	2.8	4.2	2.0	7.2	3.1	-0.8
4-fluoro- α -PPP	0.9953	0.027	0.083	7.2	-4.7	3.9	-8.2	2.3	-1.8
4'-fluoro- α -PPP	0.9987	0.058	0.176	9.2	1.3	5.6	8.4	2.3	0.1
4-APDB	0.9959	0.023	0.071	6.4	-3.4	4.3	-8.2	2.8	-1.6
4-fluoro PBP	0.9936	0.089	0.270	3.1	0.3	2.5	4.4	2.6	-0.2
5-MAPB	0.9843	0.025	0.077	6.3	-1.9	5.0	-3.7	2.3	-2.0
3-methyl BP	0.9994	0.028	0.085	8.1	-3.0	4.2	-8.1	2.7	-1.8
4-methyl- α -EAB	0.9960	0.020	0.060	7.7	-15.7	4.2	-17.8	3.8	-12.1
α -PVP metabolite 1	0.9943	0.056	0.171	8.5	-4.1	6.5	-8.1	2.6	-1.8
4-MeO- α -PVP	0.9983	0.024	0.073	2.7	-3.4	4.0	-2.1	3.3	-0.8
JWH 200 5-hydroxyindole metabolite	0.9941	0.028	0.085	8.7	-3.6	2.0	10.7	1.8	-1.9
PCMPA	0.9986	0.023	0.069	3.5	-3.3	5.2	3.6	2.5	-2.8
AM2233 azepane isomer	0.9992	0.069	0.209	6.3	-7.3	4.7	-6.8	2.8	-1.3
4-MeO PV8	0.9960	0.015	0.046	3.5	-6.5	1.8	-1.0	2.3	-0.5
Benocyclidine	0.9956	0.009	0.028	6.1	-8.0	1.9	1.1	1.6	-0.7
25I-NBMD	0.9991	0.076	0.230	6.5	-3.8	8.6	-15.8	2.0	-5.1
AB-005	0.9995	0.056	0.169	13.0	-0.4	3.4	12.4	4.3	-2.2
Flubromazepam	0.9601	0.017	0.052	11.1	-8.3	2.7	-11.0	3.4	-2.0
AM694 N-(5-hydroxypentyl) metabolite	0.9928	0.036	0.109	6.2	-7.0	4.5	4.6	2.6	-1.1
5-fluoro SDB-006	0.9934	0.015	0.047	4.2	-6.6	2.4	1.9	2.9	-0.4
PB-22 6-hydroxyisoquinoline isomer	0.9976	0.051	0.154	4.9	-12.2	5.5	-13.5	1.7	-2.1
NPB-22	0.9941	0.086	0.260	5.1	-2.3	3.6	-0.5	4.0	2.2

Table 9. LOD, LOQ, R2 values and precision and bias values for all compounds in Mix 2 at three different concentration levels.

Compound Name	R ²	LOD (ng/mL)	LOQ (ng/mL)	Low (5 ppb)		Medium (20 ppb)		High (80 ppb)	
				% CV	% Bias	% CV	% Bias	% CV	% Bias
3,4-DHMA	0.9821	0.005	0.014	2.5	4.1	2.5	-10.7	0.9	-5.6
2-fluoromethcathinone	0.9854	0.006	0.017	19.3	15.4	4.1	-8.5	0.2	-23.5
4-fluoroisocathinone	0.9515	0.018	0.052	2.8	-1.6	1.4	-6.5	2.2	-9.2
4-hydroxy MiPT	0.9798	0.015	0.045	1.3	-12.9	6.3	-2.6	1.5	-3.1
Clencyclohexerol	0.9989	0.025	0.074	1.9	-22.0	2.4	0.7	1.4	-3.9
N-Ethylbuphedrone	0.9987	0.006	0.017	1.7	0.4	1.6	-0.8	1.6	-0.7
4-MMC	0.9975	0.017	0.050	2.5	-1.5	1.4	2.4	1.8	-5.6
3-methyl-a-Pyrrolidinopropiophenone	0.9973	0.003	0.008	2.6	8.6	1.5	-2.1	2.5	-0.6
3,4-dimethoxy-a-pyrrolidinopentiophenone	0.9968	0.003	0.010	1.5	9.3	1.8	-2.8	1.6	-1.1
2,3-methylenedioxy pyrovalerone	0.9986	0.013	0.040	5.0	-1.4	5.7	-5.2	2.2	-6.1
4-ethyl-N,N-dimethylcathinone	0.9978	0.002	0.006	2.0	9.9	1.5	-0.5	2.2	-2.3
2C-T-2	0.9879	0.019	0.057	2.0	-11.4	1.7	6.3	1.9	-3.8
PCPr	0.9991	0.015	0.046	1.2	-7.1	1.1	2.9	2.6	-0.2
2C-T-4	0.9833	0.022	0.068	3.3	-13.5	1.1	11.6	1.9	-5.9
4'-methyl-N-methylhexanophenone	0.9987	0.005	0.017	2.6	3.8	1.6	0.3	1.8	-3.1
25I-NBF	0.9975	0.001	0.003	2.2	-9.5	1.4	-0.7	2.0	-4.7
Loperamide	0.9961	0.008	0.026	3.2	3.3	0.4	-7.0	1.9	-4.8
AB-005 azepane isomer	0.9980	0.007	0.020	1.5	-15.0	2.1	-4.8	1.2	-7.7
A-796260	0.9974	0.032	0.097	1.5	-7.4	1.7	4.9	1.7	-1.3
AB-FUBINACA 3-fluorobenzyl isomer	0.9985	0.008	0.024	1.0	-9.9	1.3	-5.2	2.2	-4.9
JWH 018 N-(5-hydroxypentyl) metabolite	0.9984	0.011	0.032	2.0	-6.3	2.1	2.0	1.6	-1.0
MAM2201 N-pentanoic acid metabolite	0.9987	0.012	0.035	1.1	-6.9	1.7	4.6	2.3	1.3
ADB-PINACA isomer 1	0.9992	0.009	0.028	0.7	-9.7	4.3	6.4	2.2	0.7
RCS-4 2-methoxy isomer	0.9959	0.011	0.033	2.1	-6.2	1.8	15.8	2.3	-5.5

PB-22	0.9978	0.004	0.012	1.1	-20.6	2.0	5.2	1.5	0.1
XLR11 N-(2-fluoropentyl) isomer	0.9933	0.002	0.007	7.6	-31.3	1.7	5.0	1.8	-1.0
UR-144 Degradent	0.9944	0.003	0.008	0.6	-30.8	1.8	2.1	1.7	-2.8
AKB48 N-(5-fluoropentyl) analog	0.9960	0.021	0.064	4.0	-22.6	1.2	-4.5	3.3	1.1
KM 233	0.9903	0.017	0.052	2.1	-35.4	0.8	2.4	4.5	1.6
Δ 8- THC	0.9806	0.023	0.069	7.9	-37.6	2.2	-8.4	3.5	2.7
EG-018	0.9598	0.023	0.070	1.1	-37.5	1.4	-12.0	2.4	3.6
SER-601	0.9923	0.011	0.033	1.7	-38.5	0.6	3.6	2.1	2.0

Table 10. LOD, LOQ, R² values, and precision and bias values for all compounds in Mix 3 at three different concentration levels.

Compound Name	R ²	LOD (ng/mL)	LOQ (ng/mL)	Low (5 ppb)		Medium (20 ppb)		High (80 ppb)	
				% CV	% Bias	% CV	% Bias	% CV	% Bias
N,N-dimethylcathinone	0.9858	0.02	0.06	6.8	-2.8	2.8	-1.7	3.1	4.2
Phenylpiperazine	0.9825	0.02	0.07	6.7	9.3	4.5	4.0	4.5	1.9
tetrahydro_harmine	0.9992	0.02	0.07	13.4	9.2	8.0	0.7	8.7	-2.7
MMAI	0.9987	0.02	0.07	7.9	9.0	7.9	6.6	6.4	5.5
2,3-pentylone isomer	0.9878	0.05	0.15	8.3	-0.3	3.4	-2.2	2.9	3.0
(-)-3,4-Methylenedioxy Pyrovalerone	0.9875	0.04	0.27	5.5	3.0	4.7	4.9	4.9	2.3
3C-B-fly	0.9506	0.06	0.18	7.5	3.6	4.3	5.7	8.5	10.9
para-Fluorofentanyl	0.9832	0.05	0.11	10.5	9.3	3.7	-1.2	3.6	1.0
PCEEA	0.9984	0.07	0.2	3.8	8.5	2.6	2.7	2.7	4.0
Benzylamine	0.9963	0.03	0.09	6.6	12.1	3.7	5.1	3.4	-2.1
Bromazepam	0.9463	0.04	0.13	8.8	5.7	4.3	-1.1	3.9	-0.5
AB-PINACA N-(5-hydroxypentyl) metabolite	0.9880	0.03	0.09	11.0	-0.2	3.7	2.8	2.1	2.8
25E-NBOMe	0.9840	0.01	0.03	9.8	8.6	4.0	3.0	7.3	8.3
Etaqualone	0.9915	0.04	0.12	10.6	-0.3	4.0	-3.5	3.3	6.4
JWH 198	0.9934	0.07	0.21	8.5	-13.9	37.7	-1.8	32.9	2.0
AB-FUBINACA	0.9988	0.02	0.05	4.9	8.7	3.0	2.6	3.0	3.3

(+)-WIN 55,212-2	0.9990	0.02	0.05	4.4	14.7	3.2	-1.0	4.8	3.0
JWH 073 6-methoxyindole analog	0.9956	0.03	0.09	5.0	27.8	5.5	1.9	5.5	-0.7
JWH 073 2'-naphthyl-N-(1,1-dimethylethyl) isomer	0.9968	0.12	0.36	6.4	16.9	7.4	-6.3	6.1	2.5
BB-22 8-hydroxyisoquinoline isomer	0.9979	0.04	0.13	5.2	25.9	5.1	-6.2	5.3	-4.0

4.3.4 Freeze-thaw stability

Quality control samples at concentrations of 5 and 80 ng/mL were analyzed over three different freeze-thaw cycles to determine stability. Each day a fresh set of calibrators were analyzed with the sets of samples for quantification. Samples after each thaw were compared to the concentration of time zero samples. Concentrations within $\pm 15\%$ of the time zero value were considered to be acceptable. The percent change in concentration for all compounds in Mixes 1, 2, and 3 at two different concentrations, from time zero until the 3rd thaw, can be seen in Tables 11, 12, and 13, respectively. All compounds were within the acceptable range after three freeze-thaw cycles for both concentrations, except for AB-005, etaqualone, benzydamine, 3,4-DHMA, 4-fluoroisocathinone, 4-hydroxy MiPT, 4-MMC, and a few others. The freeze-thaw stability study shows that it will be acceptable to store case work samples at -20°C for up to three freeze-thaw cycles for the majority of compounds. No information on longer term storage is provided by this work; further testing would be required to assess extended storage.

Table 11. Freeze-thaw stability for all analytes in Mix 1.

Compound Name	LOW (%Δc)	HIGH (%Δc)
4-hydroxy-MET	-12.7	-9.7
N-Methyltryptamine	-0.7	-4.2
4'-fluoro- α -pyrrolidinopropiophenone	1.7	-2.5
4-APBD	5.2	4.5
4-fluoro- α -pyrrolidinobutiophenone	0.8	-2.4
5-MAPB	-0.1	-0.6
3-Methylbuphedrone	-0.9	-3.6
4-methyl- α -ethylaminobutiophenone	-5.0	-0.9
α -Pyrrolidinopentiophenone	0.01	-0.7
4-methoxy- α -pyrrolidinopentiophenone	1.6	-0.9
PCMPA	-2.8	-1.9
AM2233 azepane isomer	-5.3	-0.5
JWH 200 5-hydroxyindole metabolite	0.7	-0.6
4-methoxy PV8	2.6	-0.4
Benocyclidine	-3.5	0.2
25I-NBMD	5.8	0.2
AB-005	-52.5	15.3
Flubromazepam	14.6	8.2
AM694 N-(5-hydroxypentyl) metabolite	-1.4	2.4
5-fluoro SDB-006	-0.3	-1.1
JWH 081 N-(5-hydroxypentyl) metabolite	-2.6	0.3
JWH 203	-3.5	0.9

Table 12. Freeze-thaw stability for all analytes in Mix 2 (LC-QqQ-MS)

Compound Name	LOW (%Δc)	HIGH (%Δc)
3,4-DHMA	-62.0	-64.6
2-fluoromethcathinone	-25.1	-20.4
4-fluoroisocathinone	-45.9	-8.2
4-hydroxy MiPT	-34.1	-33.3
Clencyclohexerol	0.7	33.9
N-Ethylbuphedrone	1.4	-8.7
4-MMC	-2.4	-63.0
3-methyl- α -Pyrrolidinopropiophenone	5.0	-3.0
3,4-dimethoxy- α -pyrrolidinopentiophenone	2.6	1.5
2,3-methylenedioxy pyrovalerone	5.8	-51.3
4-ethyl-N,N-dimethylcathinone	1.8	5.4
2C-T-2	11.3	65.8
PCPr	4.7	-32.9
2C-T-4	7.9	66.9
4'-methyl-N-methylhexanophenone	-2.7	-12.4

25I-NBF	5.7	-9.6
Loperamide	-2.7	-59.2
AB-005 azepane isomer	-2.6	-14.2
A-796260	1.1	-5.8
AB-FUBINACA 3-fluorobenzyl isomer	-2.6	-57.3
JWH 018 N-(5-hydroxypentyl) metabolite	-0.5	38.7
MAM2201 N-pentanoic acid metabolite	-5.3	-18.6
ADB-PINACA isomer 1	-17.3	46.6
RCS-4 2-methoxy isomer	6.1	2.7
PB-22	5.4	-26.2
XLR11 N-(2-fluoropentyl) isomer	-3.4	42.9
UR-144 Degradent	1.9	38.8
AKB48 N-(5-fluoropentyl) analog	3.0	28.9
KM 233	-4.9	8.3
Δ 8- THC	-4.5	-22.9
EG-018	-33.3	-51.2
SER-601	-14.1	1.9

Table 13. Freeze-thaw stability for all analytes in Mix 3 (LC-QqQ-MS)

Compound Name	LOW (% Δ c)	HIGH (% Δ c)
N,N-dimethylcathinone	-11.8	9.2
Phenylpiperazine	7.2	14.3
MMAI	19.5	21.1
2,3-pentylone isomer	14.0	16.7
(-)-3,4-Methylenedioxy Pyrovalerone	-4.1	5.0
para-Fluorofentanyl	5.6	-9.3
3C-B-fly	0.2	-1.5
PCEEA	-2.5	10.4
Benzydamine	-28.7	-18.4
Bromazepam	-5.4	-14.9
AB-PINACA N-(5-hydroxypentyl) metabolite	-2.4	-13.9
25E-NBOMe	-10.6	-20.4
JWH 198	7.5	27.0
Etaqualone	1.2	-59.0
AB-FUBINACA	4.9	2.1
(+)-WIN 55,212-2	2.0	1.7
JWH 073 6-methoxyindole analog	6.3	-3.7
JWH 073 2'-naphthyl-N-(1,1-dimethylethyl) isomer	6.7	-8.6
BB-22 8-hydroxyisoquinoline isomer	-6.7	2.7
JWH 019	-1.8	8.8

4.3.5 Matrix effects and recovery

The ME and percent recovery were determined for each analyte using the procedure previously described for LOW, MED, and HIGH concentrations. A summary of matrix effects and percent recoveries for the compounds in Mixes 1, 2, and 3 can be found in Tables 14, 15, and 16 respectively. When following OSAC guidelines, the ME for each analyte is determined using the highest value noted for the concentrations tested. Acceptable values of ME for method validation must fall within $\pm 25\%$ of the peak area of the analyte in no matrix (*i.e.*, in water). The majority of compounds at MED and HIGH concentrations fell well within these parameters with a few outliers, including 4-APBD, 4-methoxy- α -pyrrolidinopentiophenone, and AB-005. However, at the low concentration of 5 ng/mL, many of the compounds did not fall within the OSAC parameters, a finding that was not completely unexpected when working with such low concentrations. Regardless, the matrix effects experienced with these compounds did not negatively affect detection, as recovery for most compounds was above 85% at all concentration levels. A few compounds, including N-methyltryptamine and 4-APBD, showed recoveries above 100%, which likely reflects ion enhancement.

Figure 3 show how synthetic cannabinoids (n=40) vary in recovery based on their concentration, three concentration levels were analyzed low (5 ng/mL) medium (20 ng/mL) and high (80 ng/mL). As the concentration increases the percent recovery also increases. There is a statistically significant difference between medium and low and high and low concentrations. As can be seen in Figure 3, the recoveries for the lowest level vary more than the other two levels and are significantly lower. This could be due to ion suppression being higher at the lower limits of the calibration curve for the validated

method. These results relate to Figure 4, which shows a one-way ANOVA comparing the matrix effects of the same three levels for synthetic cannabinoids (n=40). It can be seen in Figure 4 that the low-level samples have more ion suppression than the higher level samples. Both ANOVAs were created only using a small subset of the total number of synthetic cannabinoids included in the final dMRM method. There is no clear pattern in terms of recovery or matrix effects for synthetic cannabinoids, which can be attributed to the there being multiple subclasses of synthetic cannabinoids.

Level	- Level	Difference	Std Err Dif	Lower CL	Upper CL	p-Value
med	low	16.57100	2.184544	11.3640	21.77800	<.0001*
high	low	14.22212	2.216655	8.9386	19.50567	<.0001*
med	high	2.34888	2.126592	-2.7200	7.41775	0.5138

Figure 3. The top panel visually represents the results of a Means/ANOVA test used to determine whether results of the recovery of synthetic cannabinoids are statistically different at three different concentration levels (5, 20, and 80 ng/mL). The bottom panel shows the results of Tukey HSD test showing that there is a statistically significant difference in terms of recovery between the medium and low levels and the high and low levels.

Level	- Level	Difference	Std Err Dif	Lower CL	Upper CL	p-Value
high (80 ng/mL)	low (5 ng/mL)	14.42069	5.601007	1.05394	27.78744	0.0314*
high (80 ng/mL)	med (20 ng/mL)	9.59236	5.650795	-3.89320	23.07793	0.2122
med (20 ng/mL)	low (5 ng/mL)	4.82833	5.650795	-8.65724	18.31389	0.6703

Figure 4. The top panel visually represents the results of a Means/ANOVA test used to determine whether or not matrix effects of synthetic cannabinoids are statistically different at three different concentration levels (5, 20, and 80 ng/mL). The bottom panel shows the results of Tukey HSD test showing that there is a statistically significant difference in terms of matrix effects between the high and low levels.

Table 14. Percent matrix effects and percent recovery for all compounds in Mix 1.

Compound Name	Low (5 ppb)		Medium (20 ppb)		High (80 ppb)	
	ME (%)	%Recovery	ME (%)	%Recovery	ME (%)	%Recovery
4-hydroxy-MET	1.4	93.7	33.9	94.9	13.3	98.5
N-Methyltryptamine	-10.5	104.2	31.7	107.2	23.9	99.2
4'-fluoro- α -pyrrolidinopropiophenone	-26.6	95.3	8.9	91.8	7.7	98.2
4-APBD	47.3	101.3	63.3	108.4	55.9	100.1
4-fluoro- α -pyrrolidinobutiophenone	-33.4	96.6	13.6	91.8	4.8	98.4
5-MAPB	-57.7	100.3	5.2	104.4	-7.2	99.8
3-Methylbuphedrone	-60.7	98.1	6.4	96.3	-18.1	98.0
4-methyl- α -ethylaminobutiophenone	-65.8	97.0	-0.8	91.9	-14.2	98.2
α -Pyrrolidinopentiophenone	-50.9	95.6	8.3	93.1	-10.5	99.6
4-methoxy- α -pyrrolidinopentiophenone	-55.2	95.9	0.2	91.9	-15.9	98.2
PCMPA	23.2	96.6	37.0	97.9	27.2	99.2
AM2233 azepane isomer	-54.0	96.4	-1.3	110.7	-13.1	98.1
JWH 200 5-hydroxyindole metabolite	-37.3	96.7	5.4	103.6	3.8	97.2
4-methoxy PV8	-40.6	92.7	7.8	93.2	-5.0	98.7
Benocyclidine	-35.1	93.5	-4.8	99.0	-8.1	99.5
25I-NBMD	-31.6	92.0	-3.5	101.1	-2.2	99.3
AB-005	-122.7	96.2	-48.3	112.3	4.9	94.9
Flubromazepam	-7.6	99.6	15.4	112.4	7.0	97.8
AM694N-(5-hydroxypentyl) metabolite	-10.2	91.7	12.2	89.0	5.2	98.0
5-fluoro SDB-006	-11.1	93.0	5.8	104.6	-2.2	98.9
JWH081N-(5-hydroxypentyl) metabolite	-12.4	93.4	10.3	101.9	2.9	99.6
JWH 203	-30.9	N/A	-43.0	99.5	-0.4	102.2

Table 15. Percent matrix effects and percent recovery for all compounds in Mix 2.

Compound Name	Low (5 ppb)		Medium (20 ppb)		High (80 ppb)	
	ME (%)	%Recovery	ME (%)	%Recovery	ME (%)	%Recovery
3,4-DHMA	-16.9	104.1	-122.2	89.3	-266.0	94.4
2-fluoromethcathinone	60.7	115.4	-25.3	91.5	-395.7	76.5
4-fluoroisocathinone	60.6	98.4	63.6	93.4	66.0	90.8
4-hydroxy MiPT	18.8	87.1	11.0	97.4	17.2	96.9
Clencyclohexerol	30.2	78.0	23.6	100.7	22.3	96.1
N-Ethylbuphedrone	36.2	100.4	29.4	99.2	-36.4	99.3
4-MMC	26.4	98.5	17.9	102.4	-11.6	94.4
3-methyl-a-Pyrrolidinopropiophenone	5.5	108.6	7.6	97.9	-11.9	99.4
3,4-dimethoxy-a-pyrrolidinopentiophenone	6.3	109.3	5.2	97.2	-14.0	98.9
2,3-methylenedioxy pyrovalerone	7.2	98.6	6.4	94.8	-12.9	93.9
4-ethyl-N,N-dimethylcathinone	14.0	109.9	6.8	99.5	-14.7	97.7
2C-T-2	19.8	88.6	10.6	106.3	-2.7	96.2
PCPr	16.5	92.9	1.9	102.9	-13.5	99.8
2C-T-4	32.1	86.5	26.3	111.6	17.7	94.1
4'-methyl-N-methylhexanophenone	24.4	103.8	14.0	100.3	-2.3	96.9
25I-NBF	11.7	90.5	-9.7	99.3	-18.9	95.3
Loperamide	1.4	103.3	-32.2	93.0	-58.6	95.3
AB-005 azepane isomer	4.0	85.0	-34.3	95.2	-67.4	92.3
A-796260	5.5	92.6	-5.3	104.9	0.3	98.7
AB-FUBINACA 3-fluorobenzyl isomer	-26.1	90.1	-50.7	94.8	-42.4	95.1
JWH 018 N-(5-hydroxypentyl) metabolite	7.7	93.8	0.5	102.0	7.1	99.0
MAM2201 N-pentanoic acid metabolite	14.7	93.1	8.6	104.6	16.3	101.3
ADB-PINACA isomer 1	8.8	90.3	2.4	106.4	5.4	100.7
RCS-4 2-methoxy isomer	6.6	93.8	-2.5	115.7	3.0	94.5
PB-22	7.0	79.4	-10.2	105.2	3.8	100.1
XLR11 N-(2-fluoropentyl) isomer	6.5	68.8	2.6	105.0	14.0	99.0

UR-144 Degradent	3.6	69.2	-1.3	102.1	14.0	97.2
AKB48 N-(5-fluoropentyl) analog	6.4	77.4	-2.0	95.5	2.9	101.1
KM 233	21.7	64.6	25.3	102.4	49.3	101.6
delta 8- THC	28.5	62.4	29.5	91.6	63.3	102.7
EG-018	17.7	62.5	25.8	88.0	51.5	103.6
SER-601	10.3	61.5	-20.1	103.6	24.9	102.0

Table 16. Percent matrix effects and percent recovery for all compounds in Mix 3.

Compound Name	Low (5 ppb)		Medium (20 ppb)		High (80 ppb)	
	ME (%)	%Recovery	ME (%)	%Recovery	ME (%)	%Recovery
N,N-dimethylcathinone	-0.9	102.8	3.8	101.7	18.1	95.8
Phenylpiperazine	28.7	90.7	19.6	96.0	37.2	98.1
MMAI	18.2	90.8	25.4	99.3	40.2	102.8
2,3-pentylone isomer	19.5	91.0	20.7	93.4	44.8	94.5
(-)-3,4-Methylenedioxy Pyrovalerone	-4.8	100.3	-7.5	102.2	15.9	97.0
p-Fluorofentanyl	-6.9	97.0	-8.8	95.1	9.6	97.7
3C-B-fly	14.3	96.4	15.9	94.3	38.1	89.1
PCEEA	4.7	90.7	7.8	101.2	20.1	99.0
Benzylamine	2.3	91.5	10.3	97.3	24.6	96.0
Bromazepam	-10.7	87.9	-13.1	94.9	-407.0	102.1
AB-PINACA N-(5-hydroxypentyl) metabolite	10.2	94.3	23.4	101.2	25.7	100.5
25E-NBOMe	-23.5	100.2	-11.5	97.3	3.4	97.2
JWH 198	-7.7	91.4	-22.9	97.0	-26.7	91.7
Etaqualone	-7.0	100.3	14.5	103.5	27.4	93.6
AB-FUBINACA	-29.8	113.9	-9.3	101.8	-14.4	98.0
-(-)-JWH 018 N-(4-hydroxypentyl)	-3.4	91.3	11.3	97.5	24.8	96.7

metabolite						
(+)-WIN 55,212-2	11.7		-182.0	85.8	-35.3	88.2
JWH 073 6-methoxyindole analog	1.4	85.3	18.9	101.0	26.4	97.0
JWH 073 2'-naphthyl-N-(1,1-dimethylethyl) isomer	-5.4	72.3	4.4	98.1	27.0	100.7
BB-22 8-hydroxyisoquinoline isomer	-6.0	83.1	9.5	106.3	29.9	97.5
JWH 019	-3.3	74.1	-2.7	106.2	32.5	104.0

4.4 Discussion

Recent research has investigated the potential of LC-QqQ-MS for the comprehensive screening, confirmation, and quantitation of NPS in human sample matrices. For example, in 2010 Wohlfarth and co-workers published an LC-MS/MS method capable of detecting over thirty NPS. Their work was one of the first attempts to create a comprehensive screening method for NPS.⁷⁸ Since then, other research groups have continued the work in the hopes of creating comprehensive screening and confirmatory methods for these substances. However, many of those methods are focused on a single structural class of NPS (*e.g.*, synthetic cannabinoids or cathinones) and can only quantitate a relatively small number of compounds.^{79,81,82,110} A recent review article on different screening methods for detecting NPS in biological matrices indicated that there were only a few methods reportedly capable of detecting >100 NPS in a single run, while the majority of methods could detect fewer than 50 compounds.¹¹⁴ In addition, some of the methods capable of detecting over 100 individual NPS were not fully validated.^{80,84}

Strickland et al. published a method that aimed to be all-inclusive for the detection of designer drugs. Strickland's research is one of the few examples of a method that includes multiple classes of NPS. Their method took 4.5 minutes and targeted 24 compounds.¹¹³ The importance of their method was in assuring that the compounds included were recently found in forensic case samples and are currently being abused. Even with that consideration, it is difficult to cover such a wide array of abused compounds with such a rapid method. Al-Saffar and co-workers also published a method that aimed to be able to detect different classes of NPS in a single run. Their method included 26 compounds, but it was validated for qualitative and quantitative analysis.¹¹⁵ Even though

their method is a move in the right direction, it excluded a large portion of abused NPS. These data confirm the need for comprehensive screening methods for NPS in biological matrices that can reliably detect the hundreds of individual chemical entities that can be considered as NPS.

One major challenge in developing comprehensive screening/confirmatory methods for hundreds of NPS involves the approach used to validate such a method. Specifically, validation using a classical “one component at a time” approach is prohibitive in terms of the time required for complete method development. In contrast, validation using non-coeluting analyte mixtures holds promise for substantially reducing the time it takes to fully validate a new method designed to encompass a large number of analytes. Validating screening/confirmatory methods using a mixture approach, while not a new concept, has only been reported on a limited basis for quantitation of NPS.⁸⁰⁻⁸² For example, Ammann and co-workers used a mixture approach to validate two separate quantitative methods, one for synthetic cathinones and another for synthetic cannabinoid each capable of confirming 25 compounds.^{79,110} Two different runs, each optimized for structural class, were required for complete analysis. In contrast, there are currently no available reports using NPS mixtures in order to validate a single comprehensive method capable of detecting multiple NPS drug classes in one run. In the present study, a LC-QqQ-MS dMRM-based assay was fully validated according to OSAC guidelines for 80 NPS using three non-coeluting mixtures of NPS standards as a proof-of-principle of the mixture approach.

Further work is underway to extend validation to additional NPS mixes to ultimately include more than 800 individual compounds. Work is also being done to test different

extraction methods for NPS from whole blood using the method discussed in the present work, showing its potential to be used to detect NPS in whole blood in addition to urine. The final validated assay could potentially have significant impact on forensic toxicology laboratories, giving them the ability to screen for a higher number of NPS than many are currently capable of. The ability to detect an increased number of NPS will decrease the number of false negatives, allowing for the proper detection of NPS. With time, more and more NPS are being introduced into the illicit market. New compounds can be added to the present assay as soon as commercial standards are produced, allowing for constantly increasing number of analytes that can be screened for.

Although the mixture approach described here has many benefits for the development of comprehensive NPS screening methods, there are also some challenges. For example, the method must be optimized for a large number of analytes in each mixture as a whole. Consequently, some individual compounds in the mixture may not be analyzed under their optimal conditions. In addition, while there is the temptation of adding new analytes to a mixture as standards become available in order to limit analysis time, this must be balanced against the loss of selectivity that can accompany the use of larger mixtures. Nevertheless, the use of analyte mixtures as described in the present work appears to be a promising approach to validate analytical methods for screening large numbers of NPS.

4.5 Conclusions

It is important to have a comprehensive screening technique for NPS in clinical and forensic toxicology laboratories in order to address the large number of NPS currently available and continuously appearing on the illicit drug market. In the present

study, a comprehensive LC-QqQ-MS method capable of screening and confirmation was developed for the detection of 729 NPS, which is by far the largest number of NPS to be included in a comprehensive analytical method to date. This was accomplished using a mixture approach in order to reduce time required for method validation. The present work demonstrates that it is possible to use a series of standard mixtures for the validation of a method containing a large number of NPS.

5. COMPARISON OF MULTIPLE EXTRACTION/PURIFICATION METHODS

5.1 Introduction

Novel psychoactive substances are compounds that are manufactured to emulate classically known and used illicit drugs. Novel psychoactive substances are often classified by one of the following drug classes; phenethylamines, amphetamines, synthetic cathinone, synthetic cannabinoids, piperazines, pipradrols/piperidines, benzofurans, and tryptamines. Clandestine laboratories circumvent federal rules and regulations by altering the structure of the parent compounds, creating new synthetic compounds, and ultimately rendering them just outside of federal jurisdiction. Once these “legal highs,” “designer drugs,” or “bath salts” are released to the public, it is not uncommon that they are followed by a wave of overdoses and potential fatalities. This phenomenon gives rise to an even greater issue for clinical and forensic toxicology laboratories; the extraction, detection, identification, and quantitation of NPS. The work presented here aims to focus on the extraction of NPS from biological matrices, which will then aid in the detection and quantitation of NPS.

The most commonly used extraction/purification techniques in forensic toxicology laboratories are “dilute-and-shoot”, protein precipitation (“crash-and-shoot”; PP), solid phase extraction (SPE) and liquid-liquid extraction (LLE). While these methods may not always be ideal for all drugs, due to time, cost, and lack of removal of possible interferences, they are well established for the extraction of common drugs of abuse. However, there is little available research focusing on the development of extraction methods with the sole purpose of extracting NPS from biological matrices. The majority of research done on the extraction of NPS focuses on the screening method rather than the

extraction technique.^{82,116} There are only a few publications that have investigated extraction of a small subset of NPS from biological matrices.^{92,117}

The goal of the present research is to compare several extraction methods to determine if any one approach is more reliable than the others for the purpose of extracting NPS from biological matrices. Blood and urine are two of the most important specimens collected in forensic cases, as they can provide accurate detail into endogenous as well as exogenous analytes present in a sample, or lack thereof. Thus, it is imperative to develop near optimal conditions by taking into consideration the amount of solvent used, pH, pK_a of analytes, and possible drug-drug interactions for the ideal extraction of analytes within complex matrices. With the constant emergence of NPS, there is an underlying ambiguity in that NPS that are similar in structure may co-elute. Co-eluting analytes may experience suppression or enhancement and can therefore not be specifically detected. In addition to more traditional extraction methods, the present research investigated the potential benefits of online SPE and “QuEChERS” (Quick, Easy, Cheap, Effective, Rugged and Safe) for the extraction of NPS from urine or whole blood.

Online SPE has the potential to greatly reduce analysis time, transfer steps, and sample handling. However, method development and optimization for online SPE is very complex, time consuming, and relies heavily on having the proper instrumentation. QuEChERS, developed by Anastassiades and co-workers, has become a reliable extraction method for pesticide analysis in agricultural and food produce industries.⁹³ Initially, QuEChERS served as an approach to extract the wide range of polar and nonpolar pesticide residues left on fruit and vegetables. The process of extraction for a single sample involves a two-step process; partitioning, followed by a dispersive-solid phase extraction (d-SPE)

cleanup.⁹³ The method's application has since become expanded to include the extraction of pollutants from complex matrix samples such as soil, sediment, and water. Furthermore, QuEChERS has in a very limited number of cases been modified to include the extraction of NPS found in biological matrices.^{117,118} The process of QuEChERS occurring in a "one-pot" process can further improve the process by cutting down on the number of steps, preparation time, cost of SPE cartridges, cleaning of glass, and use of harmful solvents. In this study, a QuEChERS method, modified to involve only a mini one-pot process, was compared to standard SPE methods and protein precipitation through the analysis of chromatographic profiles for mixes of NPS in whole-blood and urine.

5.2 Materials and Methods

5.2.1 Chemicals and Materials

Reference standards for all NPS compounds, including deuterated standards, were obtained from Cayman Chemical (Ann Arbor, MI) as the neat solid material for the majority of compounds, although some were already in solution. Optima LCMS grade MeOH, acetonitrile, DMSO, HPLC water, ammonium formate (99%), formic acid, ammonium hydroxide, HCl, glacial acetic acid, ammonium acetate, magnesium sulfate anhydrous, sodium acetate anhydrous, and sodium chloride were purchased from Fisher Scientific (Fair Lawn, NJ). Bulk sorbents of primary secondary amines (PSA) and end-capped C18 were purchased from United Chemical Technologies (Bristol, PA). Bond Elut Plexa PCX SPE cartridges were purchased from Agilent Technologies (Santa Clara, CA). Certified blank urine was purchased from UTAK Laboratories Inc. (Valencia, CA) and aliquoted into 10 mL portions and stored at -20°C until needed. Blank human whole blood

with disodium EDTA as an anticoagulant was purchased from BioIVT (Hicksville, NY) and stored at 4°C.

5.2.2 Preparation of standard solutions

Neat standards (including deuterated compounds) were dissolved in MeOH or DMSO, depending on the analyte's solubility, to achieve concentrations of 1, 2, 5, or 10 mg/mL. From these initial preparations, 10 µg/mL working solutions were prepared in MeOH for all analytes. The 10 µg/mL working solutions were used to create 200 ng/mL spiking solutions that contained up to 36 compounds. An internal standard for spiking was also created using the 10 µg/mL working solution.

5.2.3 Dilute-and-Shoot/Crash-and-Shoot Methodology

All urine and whole blood samples were spiked with the 200 ng/mL spiking solutions and the IS mix to reach the desired concentration. Dilute-and-shoot was completed by diluting spiked urine at a ratio of 1:5 with HPLC water and then directly injecting the diluted sample into the instrument for analysis. Crash-and-shoot was completed on spiked whole blood samples. This procedure consisted of adding 600 µL of cold acetonitrile (-20°C) to 200 µL of sample, vortexed for 30 sec and centrifuged for 5 min at 7000 rpm. After centrifuging, 100 µL of acidified MeOH (2% HCl) was added to the organic layer and dried down using a vacufuge (1 h at 45°C) and then reconstituted in 200 µL of MeOH for analysis.

5.2.4 The QuEChERS Methodology

A mini-one pot QuEChERS kit was developed in-house. For the one-pot method, certified drug free pooled whole blood with EDTA as an anticoagulant was spiked with drug mix and internal standard mix to reach a total sample volume of 0.2 mL. Before the

addition of sample, 600 μ L of cold acetonitrile and 200 μ L of HPLC water were added to a pre-weighed mini QuEChERS kit consisting of 200 mg of MgSO_4 , 50 mg of NaCl, 25 mg of PSA, 25 mg of end-capped C_{18} , and ceramic homogenizer beads. Next, 200 μ L of sample was added to the kit then the sample was shaken by hand for 30 s, vortexed for 1 min, and centrifuged for 5 min at 10,000 rpm. Acidified MeOH was added to the supernatant before being dried down using a vacufuge. Once dry, the sample was reconstituted in 200 μ L of MeOH before LC-MS/MS analysis.

5.2.5 On-line SPE Methodology

Online SPE was initially performed using an Agilent 1290 Infinity Online SPE Solution in conjunction with an Agilent 1290 FlexCube LC unit, with a Bond Elute (BE) online polymeric sorbent material (PLRP-S) cartridge. An online SPE method was created by altering the dMRM method that had been previously developed for the detection of NPS. The online SPE method included parameters for the Agilent Flex Cube LC unit using the same mobile phases as those developed for the screening method (*i.e.*, initial 95:5 A:B; final 2:98 A:B mobile phases).

After initial experiments using this online SPE method were unsatisfactory, a new approach was taken in order to increase the retention and recovery of all compounds. This revised online SPE method utilized two different cartridges which were loaded and eluted at the same time. For this purpose, the BE cartridge described above was used in tandem with a mixed mode cartridge. This approach was developed in the hope of reducing tailing and peak broadening encountered with the initial method. It was reasoned that a mixed mode cartridge could allow for some the compounds to elute faster rather than being

retained on the reverse phase column. To properly load and elute using the two cartridges, modified FlexCube parameters were developed (Table 17).

Table 17. Programming timetable for the Flex Cube for Online SPE.

Time (min)	Function	Parameter
0	Pump for volume	Pump 3 mL: Flow at 0.5 mL/min
1.00	Valve change position	Position 2 (Load 2 Elute 1)
2.00	Valve change position	Position 1 (Load 1 Elute 2)
12.5	Pump for volume	Pump 3 mL: Flow at 0.5 mL/min
14.00	Pump for volume	Pump 4 mL: Flow at 0.5 mL/min

5.2.6 SPE Methodology

An SPE method previously created in the lab was altered in order to be used for a wide variety of NPS. Crash-and-shoot, as described above, was completed on 200 μ L of sample, then 1 mL of 0.1 M phosphate buffer (pH=6) was added to the organic layer. A mixed mode Plexa PXR cartridge was used for SPE. The cartridge was conditioned with 1 mL of MeOH and 1 mL of phosphate buffer. After conditioning, the sample was loaded onto the cartridge slowly and then washed with 3 mL of buffer and 3 mL of MeOH:H₂O (20:80). After washing, the cartridge was dried for 10 min before elution. The sample was eluted with two 0.5 mL aliquots of MeOH:MeCN (50:50) and one 0.5 mL aliquot of 5% ammonium hydroxide in MeCN. Acidified MeOH (2% HCl) was added to the extract before drying using a vacufuge. Once dried, samples were reconstituted in 200 μ L of MeOH before analysis.

5.2.7 Comparison of techniques

Results for all extraction methods except online SPE were compared with regard to matrix effects, recovery, process efficiency, cost, and time. Recovery (RE), matrix effects (ME), and process efficiency (PE) were determined using three sample sets, all with a final NPS concentration of 50 ng/mL. The three sets were designed as follows; neat drug

dissolved in MeOH (set A), drug spiked into the sample after extraction (set B), and drug spiked into the sample before extraction (set C). Matrix effects, recovery, and process efficiency were determined according to equations 7, 8, and 9, respectively. Cost was determined based on the materials needed to run 50 samples a day for a full year and the time required per batch of samples (20 samples).

$$ME = \frac{Set\ B}{Set\ A} * 100 \quad (7)$$

$$RE = \frac{Set\ C}{Set\ B} * 100 \quad (8)$$

$$PE = \frac{Set\ C}{Set\ A} * 100 \quad (9)$$

Techniques were separated by matrix (*i.e.*, urine or whole blood) and compared accordingly. To determine whether results for any of the techniques were significantly different from one another, a one-way analysis of variance (ANOVA) was completed using JMP software version 14. An independent one-way ANOVA was done for each parameter (ME, RE, and PE) resulting in three ANOVAs for each matrix. Average peak area was used to determine ME, RE, and PE for each compound. Each of the three ANOVAs were created for all compounds in Mix 2 (n=33). An individual ANOVA was not completed per compound, instead ANOVAs were separated based on technique and parameter (ME, RE, PE). An ANOVA is only capable of determining if any of the techniques are significantly different (determined using the F ratio on the basis of sum of squares) not which specific techniques are different from one another. To determine which specific extraction techniques produced significantly different results, a Tukey's honestly significant difference (HSD) test was completed using JMP software. Cost and time were compared

subjectively and are highly dependent on the needs of the analysis (*i.e.*, whether the elimination of matrix effects is more important than recovery).

5.2.8 LC-QqQ-MS analysis

All samples were analyzed using an Agilent 1290 Infinity Binary Pump LC coupled to an Agilent 6460 triple quadrupole MS/MS with Jet Streaming technology and electrospray ionization (ESI) equipped with Agilent MassHunter software version B7.0. Chromatographic separation was performed using an Agilent Zorbax Rapid Resolution HD Eclipse Plus C₁₈ column (3.0 x 100 mm, 1.8 µm). Data acquisition was performed in dMRM mode using positive mode ESI. The dMRM method employed two transitions for each analyte, which aids in achieving increased selectivity.

Chromatographic separation was achieved using gradient elution with a flow rate of 0.3 mL/min using 5 mM ammonium formate/0.1% formic acid in HPLC water as mobile phase A and MeOH with 0.1% formic acid as mobile phase B. The gradient used for analysis was as follows: hold at 5% B for 1 min, followed by 5% B to 98% B from 1 to 9.5 min, then hold at 98% B until 16 min, followed by a 3-min re-equilibration at 5% B. The analytical column was held at a temperature of 40°C during separation.

MS source parameters were as follows: gas temperature, 325°C; gas flow 6 L/min; nebulizer 40 psi; sheath gas temperature 350°C; sheath gas flow 11 L/min; capillary voltage 4,000 V; and nozzle voltage 750 V. Agilent MassHunter Optimizer software was used to determine the ideal data acquisition parameters for MRM mode. The software uses the mass of each compound to determine optimal fragmentor voltage, resulting product ions, and associated collision energies and can optimize for up to 10 transitions for each

precursor ion. Analyte detection windows ranged from 0.25 min (*i.e.*, ± 0.125 min around t_R) to 0.75 min (*i.e.*, ± 0.375 min around t_R) depending on the analyte.

5.3 Results and Discussion

5.3.1 Dilute-and-shoot/crash-and-shoot methodology

Dilute-and-shoot was determined to be an economical and fast method, however, it exhibited the highest ion suppression/enhancement from matrix effects. This was expected, since no matrix effects were being eliminated by using this technique, just reduced via dilution. Dilute-and-shoot can be used when high throughput is necessary, since it saves both time and money, however it could cause increased instrument down time if proper cleanup precautions are not used.

Crash-and-shoot showed similar results to those of dilute-and-shoot, since it is also a minimal purification technique. The goal of crash-and-shoot is to eliminate proteins by precipitating them out using cold solvent. For many screening purposes in forensic laboratories, crash-and-shoot may be desirable to cut down on time and cost of analysis.

The results for ME, RE, PE for all compounds in Mix 2 for crash-and-shoot can be seen in Table 18. Matrix effects should ideally be 80 - 120%; there were a number of compounds for which ME was not within this range. However, the majority of compounds did exhibit ME within 60 - 85%, indicating some degree of ion suppression. This result may not be surprising, as whole blood is a complicated matrix and eliminating all matrix effects simply by removing proteins present in the sample is not always enough to sufficiently eliminate matrix effects.

Table 18. Matrix effects, recovery, and process efficiency for Mix 2 compounds in spiked (50 ng/mL) whole blood samples following crash-and-shoot processing.

Compound	ME (%)	RE (%)	PE (%)
3,4-DHMA	134	33	44
2-fluoromethcathinone	97	81	78
4-fluoroisocathinone	74	85	63
4-hydroxy MiPT	0	48	0
Clencyclohexerol	44	73	32
N-Ethylbuphedrone	79	87	69
4-MMC	88	82	72
3-methyl- α -Pyrrolidinopropiophenone	88	83	73
3,4-dimethoxy- α -Pyrrolidinopentiophenone	89	83	74
2,3-methylenedioxy pyrovalerone	89	83	74
4-ethyl-N,N-dimethylcathinone	88	84	74
2C-T-2	61	76	46
PCPr	87	81	71
2C-T-4	61	74	45
4'-Methyl-N-methylhexanophenone	88	83	77
25I-NBF	87	83	72
Loperamide	89	81	72
AB-005 azepane isomer	58	94	55
AB-FUBINACA 3-fluorobenzyl isomer	80	73	58
A-796260	55	68	37
JWH 018 N-(5-hydroxypentyl) metabolite	61	88	54
MAM2201 N-pentanoic acid metabolite	47	112	53
ADB-PINACA isomer 1	73	80	58
RCS-4 2-methoxy isomer	70	81	57
PB-22	75	71	53
XLR11 N-(2-fluoropentyl) isomer	42	73	31
UR-144 Degradant	1	62	1
AKB48 N-(5-fluoropentyl) analog	13	49	6
KM 233	1	200	2
Δ 8-THC	2	314	5
EG-018	34	73	25
SER-601	101	35	35

5.3.2 The QuEChERS Methodology

The QuEChERS method underwent some efforts at optimization before a final in-house method was created and used for the comparison work. At first, a two-step approach using a commercial (Agilent Technologies, Inc.) QuEChERS kit designed for the extraction of 2 mL samples was tested and then compared to the results of an in-house mini QuEChERS kit that was developed. Figure 5 shows the comparison of chromatograms for the one-pot approach and the two-step method when applied to whole blood samples spiked at the 5 ng/mL level. Results of the two approaches were compared based on peak area and recovery (determined using a daily calibration curve). It was determined that at lower concentration levels the in-house mini QuEChERS approach resulted in higher recoveries than the commercial two-step approach. Other concentrations (20 and 80 ng/mL) were also compared, however, there was no significant difference between the two approaches for higher concentrations.

Figure 5. The top panel represents the MRM results of extracting the compounds in Mix 2 from whole blood using an in-house one-pot approach, while the bottom panel is the result of extracting the same compounds from whole blood using a two-step approach.

Figure 6 represents the comparison of the one-pot approach and the two-step approach for QuEChERS in terms of extracting a mix of NPS from whole blood. There was no statistically significant difference between the approaches, however, there was a

wider spread of results for the two-step approach than the one-pot method. The variation of results for the two-step approach can be attributed to the low recoveries of 5 ng/mL samples in comparison to the results seen with the one-pot approach. Figure 6 shows chromatograms for the one-pot approach and the two-step approach for 5 ng/mL samples. All work moving forward was completed using the one-pot approach.

Figure 6. Visually represents the results of a Means/ANOVA test used to determine whether or not there is a statistically significant difference between the commercial two step approach and the in-house one-pot approach in terms of recovery.

Subsequently, attempts were made to further optimize the one-pot mini QuEChERS approach. Ratios and amounts of solids were modified to assess the effect on reduction of matrix effects and drug recovery. However, these efforts did not result in significant additional improvement in these parameters. Figure 7 represents the elimination of matrix effects by changing salts using QuEChERS. In the figure on the x-axis B=blood U=urine and the number correspond to the amount of MgSO_4 in mg in the QuEChERS kit. Since there were no statistically significant differences found through the one-way ANOVA and

Tukey's HSD test, the original amount of salts (150 mg) were used to create one-pot QuEChERS kits for extraction.

Level	- Level	Difference	Std Err Dif	Lower CL	Upper CL	p-Value
B 200	U 150	9.484481	6.075284	-6.4401	25.40911	0.4063
B 200	B 150	8.312173	6.274526	-8.1347	24.75906	0.5499
U 200	U 150	7.793373	6.001192	-7.9370	23.52379	0.5664
U 200	B 150	6.621065	6.202815	-9.6378	22.87998	0.7102
B 200	U 200	1.691108	6.202815	-14.5678	17.95002	0.9929
B 150	U 150	1.172308	6.075284	-14.7523	17.09694	0.9974

Figure 7. The top panel visually represents the results of a Means/ANOVA test used to determine whether or not the amounts of salts used in the QuEChERS one-pot kit has an effect on elimination of matrix effects. The bottom panel shows the results of Tukey HSD test showing that there are no statistically significant differences.

The results for ME, RE, PE for all compounds spiked into whole blood and extracted by QuEChERS can be seen in Table 19. Matrix effects observed for all compounds in Mix 2 were quite variable; the majority were below 100%, meaning that the compounds were experiencing ion suppression. Most of the compounds fall between 60 and 100% matrix effects, therefore they were experiencing up to 40% ion suppression. While there was a large range of recoveries, the majority of compounds were recovered at levels above 60%. It is not uncommon to see such varied recoveries due to the different structural classes of drugs that are being extracted, all of which have different chemical

interactions. Process efficiency on average fell between 40 and 60% for the compounds included in Mix 2. Ideally, process efficiency should be higher and the QuEChERS approach could be further optimized specifically to increase PE values.

Table 19. Matrix effects, recovery, and process efficiency for Mix 2 compounds in spiked (50 ng/mL) whole blood samples following QuEChERS processing.

Compound	ME (%)	RE (%)	PE (%)
3,4-DHMA	105	8	9
2-fluoromethcathinone	73	69	50
4-fluoroisocathinone	91	116	106
4-hydroxy MiPT	0.1	51	0.1
Clencyclohexerol	61	69	42
N-Ethylbuphedrone	63	72	45
4-MMC	69	69	47
3-methyl- α -Pyrrolidinopropiophenone	68	64	44
3,4-dimethoxy- α -Pyrrolidinopentiophenone	69	69	48
2,3-methylenedioxy pyrovalerone	68	66	45
4-ethyl-N,N-dimethylcathinone	68	61	42
2C-T-2	17	206	34
PCPr	67	61	41
2C-T-4	21	190	41
4'-Methyl-N-methylhexanophenone	68	64	44
25I-NBF	68	72	49
Loperamide	68	85	57
AB-005 azepane isomer	43	99	42
AB-FUBINACA 3-fluorobenzyl isomer	66	86	56
A-796260	31	139	43
JWH 018 N-(5-hydroxypentyl) metabolite	57	87	51
MAM2201 N-pentanoic acid metabolite	56	92	51
ADB-PINACA isomer 1	61	78	47
RCS-4 2-methoxy isomer	57	84	48
PB-22	56	84	47
XLR11 N-(2-fluoropentyl) isomer	46	87	39
UR-144 Degradant	16	79	13
AKB48 N-(5-fluoropentyl) analog	22	69	15
KM 233	5	112	6
Δ 8-THC	3	202	7
EG-018	43	57	24
SER-601	71	78	55

5.3.3 Online SPE methodology

During the online SPE method development and optimization experiments, initial results were less than ideal, resulting in severe tailing, peak broadening, and carry over. An example of a typical chromatogram collected using the one cartridge approach can be seen in Figure 8. One of the major issues faced when trying to optimize an online SPE approach for the Agilent FlexCube instrumentation was the inability to manipulate pH in the same way as that for a classical SPE approach. In order to get around this limitation, a two-cartridge approach was attempted, but also without success. It was ultimately determined that a different online SPE instrumentation design would be required to successfully extract different classes of NPS from biological matrices in just one run. For

Figure 8. Typical LC-QqQ-MS MRM response for Mix 2 using the reversed phase online SPE approach.

example, Lehman and co-workers developed an in-line SPE method for the extraction of 74 NPS from serum. Their in-line SPE set up was capable of up to 100 μ L injection volumes and was capable of higher ranges of pH manipulation. Additionally, an ideal online SPE setup would allow for higher flow rates and the ability to use more than three solvents for the SPE process. Higher flow rates would aid in the washing of impurities from the cartridge and elution of compounds of interest. The more solvent attachments allow for a method with more steps, which may be necessary to extract such varied compounds in one run. Consequently, online SPE was not considered further when

completing comparison tests for extraction/purification methods. As an alternative, classical SPE was tested and compared to the other extraction/purification methods.

5.3.4 Solid phase extraction methodology

The classical SPE approach was adapted from a previously developed approach in the lab so that it was capable of extracting different classes of NPS. The SPE method was specifically designed to extract drug compounds with varying pKa values through pH manipulation and multiple elution steps. Table 20 shows the ME, RE, and PE for all compounds included in Mix 2. The majority of compounds underwent ion some suppression in terms of matrix effects, however, most of the compounds were found to have ME above 70%, which is desired when working with whole blood. It was shown that SPE is capable of removing a large portion of matrix effects for the majority of compounds. The major exceptions were synthetic cannabinoids, which tend to pose issues when extracting from biological matrices in general. In terms of recovery, the results were generally lower than desired; RE fell within the range of 50-100%, which might be improved with further optimization. Process efficiency varied from compound to compound without a specific trend.

Table 20. Shows the results for mix 2 using SPE in terms of matrix effects, recovery, and process efficiency using 50 ng/mL spiked whole blood samples.

Compound	ME (%)	RE (%)	PE (%)
3,4-DHMA	132	3	4
2-fluoromethcathinone	23	106	24
4-fluoroisocathinone	29	64	18
4-hydroxy MiPT	69	2	1
Clencyclohexerol	85	22	19
N-Ethylbuphedrone	60	72	43
4-MMC	59	62	37
3-methyl- α -Pyrrolidinopropiophenone	77	53	41
3,4-dimethoxy- α -Pyrrolidinopentiophenone	86	55	47
2,3-methylenedioxy pyrovalerone	78	55	43

4-ethyl-N,N-dimethylcathinone	71	65	46
2C-T-2	84	11	9
PCPr	85	43	37
2C-T-4	87	13	11
4'-Methyl-N-methylhexanophenone	60	65	39
25I-NBF	85	52	44
Loperamide	88	23	20
AB-005 azepane isomer	86	54	46
AB-FUBINACA 3-fluorobenzyl isomer	95	62	59
A-796260	82	61	50
JWH 018 N-(5-hydroxypentyl) metabolite	86	78	67
MAM2201 N-pentanoic acid metabolite	93	79	73
ADB-PINACA isomer 1	86	64	55
RCS-4 2-methoxy isomer	75	74	56
PB-22	77	61	47
XLR11 N-(2-fluoropentyl) isomer	55	75	42
UR-144 Degradant	31	88	27
AKB48 N-(5-fluoropentyl) analog	15	128	19
KM 233	8	120	10
Δ 8-THC	31	79	24
EG-018	33	71	23
SER-601	93	77	72

5.3.5 Comparison of techniques

All extraction methods were compared on the basis of matrix effects, recovery, process efficiency, time, and overall cost. Table 21 shows the breakdown of how much each extraction technique would cost to analyze 20 samples including consumables, solvents, cartridges, and operator time assuming a \$22 hourly salary.. Additionally, Table 21 shows the time each method would take to extract 20 samples. It is important to consider overall cost and time when determining which extraction method is ideal for a specific purpose. However, time and cost should not be the only factors to consider in making a final decision. These parameters should be considered in addition to ME, RE, and PE.

Table 21. Cost of each extraction technique and the time each one takes to prepare a set of 20 samples.

Technique	Cost per set (\$)	Time (min)
Dilute-and-shoot (urine)	5	10
Crash-and-shoot (blood)	39	100
QuEChERS (blood)	47	120
QuEChERS (urine)	47	120
Solid phase extraction (blood)	146	210
Solid phase extraction (urine)	132	180

In addition, extraction method performance was statistically compared using a one-way ANOVA based on ME, RE, and PE, using peak area as a measure of response. The ANOVA and results of the Tukey's HSD test can be seen in Figures 9, 10, and 11.

Figure 9 The top panel visually represents the results of a Means/ANOVA test used to determine whether results of the three extraction methods based on matrix effects are significantly different. The bottom panel shows the results of Tukey HSD test showing that there is a statistically significant difference between the results of SPE and QuEChERS with blood in terms of elimination of matrix effects.

Figure 10. The top panel visually represents the results of a Means/ANOVA test used to determine whether results of the three extraction methods based on recovery are significantly different. The bottom panel shows the results of Tukey HSD test showing that there is a statistically significant difference between the results of SPE and QuEChERS with blood and crash and shoot and SPE in terms of recovery.

Figure 11. The top panel visually represents the results of a Means/ANOVA test used to determine whether results of the three extraction methods based on process efficiency are significantly different. The bottom panel shows the results of Tukey HSD test showing there are no statistically significant differences.

The one-pot QuEChERS approach when completed for urine samples showed a greater elimination of matrix effects than dilute-and-shoot, but lower elimination of matrix effects than SPE. Even though QuEChERS is capable of removing a higher level of matrix effects from urine as compared to dilute-and-shoot, it has comparable recoveries and process efficiency to dilute-and-shoot. QuEChERS for whole blood showed comparable matrix effects, recovery and process efficiency as compared to crash-and-shoot. While at first glance QuEChERS may appear capable of eliminating more matrix effects based on the ANOVA plot, the p values indicate no statistically significant differences.

The SPE approach, when completed for urine samples, showed much higher elimination of matrix effects than either dilute-and-shoot or QuEChERS. Even though SPE is capable of removing a higher level of matrix effects from urine samples than dilute-and-shoot and QuEChERS, there was no significant difference when considering recovery and process efficiency. Solid phase extraction for whole blood, like the results for urine, showed higher elimination of matrix effects than the other two extraction methods. However, SPE had lower recovery than both QuEChERS and crash-and-shoot. All three extraction methods for whole blood were comparable when looking at process efficiency. SPE is more efficient at eliminating matrix effects than the other options, but is the most time consuming and expensive. The cost and time it takes to complete SPE needs to be weighed against the need for the elimination of matrix effects.

When considering the results of the ANOVAs for all parameters and Tukey HSD tests, there is no clear answer on which technique would be most beneficial for the extraction of NPS. In order to decide which approach is appropriate, it is therefore

important to consider the overall needs of the analysis. For qualitative purposes, dilute-and-shoot and crash-and-shoot may be appealing since they are quick and cost effective. For qualitative analysis, the elimination of matrix effects is not imperative, therefore it is not necessary to use a more time and cost consuming method. If quantification is needed, then a technique capable of minimizing matrix effects would be more effective. Therefore, SPE may be the best choice for quantitation or when low limits of detection are needed. QuEChERS may be ideal when high throughput and quantification is desired due to the time difference between SPE and QuEChERS.

Finally, the drying down process was analyzed to ensure that it was not contributing to sample loss. Although this step is not necessary for all samples, it was done in this study so that all extracts had the same composition and volume for analysis. Results indicated that the drying down process does account for some analyte loss. For most compounds, there was ~10% loss of analyte in the drying process using the vacuum centrifuge. However, some compounds showed a much higher sample loss, for example 2C-T-4 and KM 233, which had ~90% loss of analyte through the drying process. To avoid potentially high sample losses from drying down, it is also possible to use a gentle stream of nitrogen as an alternative. Ideally, the drying down process should only be used when necessary, such as when the goal is to concentrate samples before analysis.

5.4 Conclusions

Various extraction methods, including dilute-and-shoot, crash-and-shoot, SPE, and QuEChERS, were analyzed and compared based on elimination of matrix effects, and improved recovery and process efficiency. It was determined that none of the methods are clearly statistically better than the others for the extraction of NPS from

urine and whole blood. Each extraction method was applied to a mixture of 33 NPS that included compounds from various drug classes and metabolites. When analyzing the mixture as a whole it was determined that SPE is capable of eliminating the most matrix effects, however SPE had the lowest recoveries. Ultimately, when deciding which extraction method is best, it is necessary to consider the goals of the final method. Further optimization would need to be completed in order to have one method that is best in all aspects for multiple drug classes. However, QuEChERS has the potential to be that method with additional optimization.

6. ANALYSIS OF BLIND SPIKES AND AUTHENTIC SPECIMENS

6.1 Introduction

Full method validation and applicability of extraction methods generally need to be tested on blind spikes and authentic specimens in order to prove adaptability to forensic case work samples.¹¹¹ A number of methods that have been validated for the detection of NPS have been applied to forensic case work samples.^{78,82} Authentic specimens have challenges that are not faced when analyzing spiked samples. Some of these challenges include interferences from licit medications, unknown concentrations of analytes, and increased matrix effects that can be caused by medical issues.

The present study included the analysis of blind spiked urine and whole blood samples and authentic ante-mortem urine specimens using the validated dMRM method for the detection of NPS. Blind spikes were qualitatively and quantitatively analyzed in order to further validate the dMRM method. Additionally, authentic specimens were qualitatively screened for all 826 analytes included in the full dMRM method. Analyzing blind spikes and authentic specimens is important in order to prove the applicability of a developed method to clinical and forensic samples.

6.2 Materials and Methods

6.2.1 Chemicals

Reference standards for all NPS compounds, including deuterated standards, were obtained from Cayman Chemical (Ann Arbor, MI) as the neat solid material for the majority of compounds, although some were already in solution. Optima LCMS grade methanol, acetonitrile, HPLC water, ammonium formate (99%), formic acid, magnesium sulfate anhydrous, sodium acetate anhydrous, and sodium chloride were purchased from

Fisher Scientific (Fair Lawn, NJ). Bulk sorbents of primary secondary amines (PSA), endcapped C18, and beta-glucuronidase were purchased from United Chemical Technologies (Bristol, PA). Certified blank urine was purchased from UTAK Laboratories Inc. (Valencia, CA) and aliquoted into 10 mL portions and stored at -20°C until needed. Blank human whole blood with disodium EDTA as an anticoagulant was purchased from BioIVT (Hicksville, NY) and stored at 4°C.

6.2.2 Collection of Authentic Specimens

Authentic urine specimens that had originally been collected in 2014 were obtained from a local drug testing laboratory, with volumes varying from 2.5 to 6.0 mL. Specimens were obtained from subjects in addiction treatment and pain medication monitoring programs and were supplied deidentified with no subject information provided. Specimens were assigned a sequential ID number for laboratory tracking purposes and were stored in a -20°C locked freezer until analysis.

6.2.2 Preparation of samples

Blind spiked urine samples were created such that identity and concentration were unknown to the analyst. Samples were created in certified blank urine at a final volume of 200 µL. Analytes were selected from Mixes 1, 2, and/or 3 and samples contained 0 to 9 individual analytes at varying concentrations. An aliquot of internal standard mix was added to each sample, which was then diluted to 1 mL with HPLC water before analysis.

Blind spiked whole blood samples were also created in the same manner as the blind spiked urine samples, *i.e.*, in certified blank human whole blood in a final volume of 200 µL. Analytes were selected from Mixes 1, 2, and/or 3 and samples contained 0 to 2 individual analytes. An aliquot of internal standard mix was added to each sample in the

first set of blind spiked whole blood samples before undergoing mini one-pot QuEChERS extraction. After extraction, the organic layer was removed and dried down using vacufuge after the addition of acidified MeOH. Once completely dry, samples were reconstituted with 200 μ L of MeOH for analysis. A second set of blind spiked whole blood samples were prepared using crash-and-shoot processing. An aliquot of internal standard mix was added to all samples before the addition of 600 μ L of cold MeCN. Once the MeCN was added, all samples were vortexed and then centrifuged. After centrifugation, the supernatant was removed and dried down using the same technique as the samples prepared using QuEChERS.

In addition, 50 authentic urine specimens were analyzed using the validated dMRM method. Authentic specimens were prepared by adding internal standard mix to 100 μ L of sample and diluting it to 500 μ L with HPLC water for analysis. After this initial analysis, the 50 authentic specimens were glucuronidase treated, making it possible to detect metabolites that may be missed otherwise. The glucuronidase solution was prepared using 2 mL of hydrolysis buffer, 18 mL water, and 5 mL of β -glucuronidase. Treatment was done by adding the glucuronidase solution to authentic urine specimens at a ratio of 1:1 and incubating for 2 h at 35°C before LC analysis. These samples were qualitatively screened for the presence of any of the 826 compounds included in the NPS standard mixes. Qualitative analysis for the blind spiked and authentic specimens was completed using the dMRM method described previously. Any peak with a signal 3 times greater than the noise was considered to be positive for that analyte. Quantitative analysis for blind spiked samples was completed using the fully validated dMRM method described previously, using a daily calibration curve and MassHunter Quantitative software.

6.3 Results and Discussion

A total of 38 blind spiked urine samples were prepared in three different sets for qualitative and quantitative analysis. The first set was designed to test the lower limits of the validated method, the second set was designed in the middle of the calibration curve, and the third was designed to test selectivity. The experimental identity, experimental concentration, true identity, and true concentration for the 15 blind spikes included in the first set are shown in Table 22. The only sample that was not properly identified was sample 1, which should have been identified as THCA-A, but was identified as JWH 200 5-hydroxyindole metabolite. This was likely due to the fact that THCA-A was one of the few compounds that did not show linearity and therefore this method was not capable of properly identifying that compound. In addition, there were three false negative results (samples 2, 4, and 6), *i.e.*, samples that were determined to be blank even though they each contained one compound, and one sample (sample 5) that contained two compounds with only one correctly identified. The false negatives could have been caused by ion suppression, which may have resulted in low ion intensity and levels that were not within the LOD/LOQ of the dMRM method, since this set of spikes was designed to test the lower limits of the method. Differences between the spiked concentration and the detected concentration may also be attributed to ion suppression or enhancement. Additionally, the use of IS not chemically identical to every analyte can also introduce errors in quantitation, due to differences in relative ionization or other factors.

Table 22. True identity and concentration and experimental identity and concentration of blind spiked urine samples included in set 1 (low concentration)

#	Compound Spiked	Conc. spiked (ng/mL)	Compound Detected	Conc. Detected (ng/mL)
1	THCA-A	5	JWH 200 5-hydroxyindole metabolite	N/A
2	4-APDB	5	nd	-
3	JWH 200 5-hydroxyindole metabolite	5	JWH 200 5-hydroxyindole metabolite	7
4	N-Methyltryptamine	5	nd	
5	3-Methylbuphedrone	5	3-Methylbuphedrone	7
	5-fluoro SDB-006	5	nd	
6	2-fluoromethcathinone	5	nd	
7	2C-T-4	5	2C-T-4	1
8	PB-22	5	PB-22*	9
9	4-ethyl-N,N-dimethylcathinone	5	4-ethyl-N,N-dimethylcathinone	3
10	JWH 018 N(5-hydroxypentyl) metabolite	5	JWH 018 N(5-hydroxypentyl) metabolite	9
	MAM2201 N-pentanoic acid metabolite	5	MAM2201 N-pentanoic acid metabolite	5
11	25E-NBOMe	5	25E-NBOMe	5
12	AB-FUBINACA	5	AB-FUBINACA*	3
13	3C-B-fly	5	3C-B-fly	4
14	Para-Fluorofentanyl	5	Para-Fluorofentanyl	3
15	N,N-dimethylcathinone	5	N,N-dimethylcathinone	1
	Phenylpiperazine	5	Phenylpiperazine	3

*Isomer of this compound were also detected.

nd- not detected.

The true identity, true concentration, experimental identity, and experimental concentration can be seen in Table 23 for all compounds in the second set of spike urine samples. This set was spiked with 0 to 2 compounds each. The only sample that was not properly identified was sample 1, which should have been identified as THCA-A, but was not. This was likely due to the fact that THCA-A was one of the few compounds that did not show linearity and therefore this method was not capable of properly identifying that compound. Virtually all of the blind spikes were correctly identified, with quantitative results generally within ± 20 of nominal. However, this method is not capable of discerning isomers and, as noted in the table, some isomers were determined in addition to the correct identification.

The true identity, true concentration, experimental identity, and experimental concentration can be seen in Table 24 for all compounds in the third set of spike urine compounds. This set was included 3 to 9 compounds per sample at varying concentrations. All compounds were identified correctly, except for THCA-A and 4'-fluoro-a-pyrrolidinopropiophenone. Again, THCA-A does not show linearity and 4'-fluoro-a-pyrrolidinopropiophenone was eliminated due to low abundance, which may have been caused by ion suppression. This set was designed to test selectivity, however, samples were still quantified and tested the full linear range of the method.

Table 23. The true identity and concentration and experimental identity and concentration of blind spiked urine samples included in set 3 (intermediate concentration)

#	Compound Spiked	Conc. spiked (ng/mL)	Compound Detected	Conc. Detected (ng/mL)
1	THCA-A	50	Benzydamine	-
2	4-APDB	50	4-APDB	57
3	JWH 200 5-hydroxyindole metabolite	50	JWH 200 5-hydroxyindole metabolite	45
4	blank		None	-
5	N-Methyltryptamine	50	N-Methyltryptamine	43
6	3-Methylbuphedrone	50	3-Methylbuphedrone	56
	5-fluoro SDB-006	50	5-fluoro SDB-006	61
7	2-fluoromethcathinone	50	2-fluoromethcathinone	47
8	2C-T-4	50	2C-T-4	60
9	PB-22	50	PB-22*	70
10	blank		None	-
11	4-ethyl-N,N-dimethylcathinone	50	4-ethyl-N,N-dimethylcathinone	42
12	JWH 018 N(5-hydroxypentyl) metabolite	50	JWH 018 N(5-hydroxypentyl) metabolite*	55
	MAM2201 N-pentanoic acid metabolite	50	MAM2201 N-pentanoic acid metabolite	44
13	25E-NBOMe	50	25E-NBOMe	45
14	AB-FUBINACA	50	AB-FUBINACA*	65
15	3C-B-fly	50	3C-B-fly	57
16	para-Fluorofentanyl	50	para-Fluorofentanyl	46
17	blank		None	-
18	N,N-dimethylcathinone	50	N,N-dimethylcathinone	45
	Phenylpiperazine	50	Phenylpiperazine	53

*Isomers of these compounds were also detected

Table 24. The true identity and concentration and experimental identity and concentration of blind spiked urine samples included in set 2 (varied concentrations)

#	Compound Spiked	Conc. Spiked (ng/mL)	Compound Detected	Conc. Detected (ng/mL)
1	Benocyclidine	5	Benocyclidine	5
	JWH 073 2'-naphthyl-N-(1,1-dimethyl) isomer	5	JWH 073 2'-naphthyl-N-(1,1-dimethyl) isomer	6
	4-hydroxy MiPT	5	4-hydroxy MiPT	6
	JWH 200 5-hydroxyindole metabolite	35	JWH 200 5-hydroxyindole metabolite	36
	XLR11 N-(2-fluoropentyl) isomer	35	XLR11 N-(2-fluoropentyl) isomer	32
	PB-22	35	PB-22*	70
	MMAI	35	MMAI	32
	4-methoxy PV8	80	4-methoxy PV8	77
	2-fluoromethcathinone	80	2-fluoromethcathinone	50
2	N-Ethylbuphedrone	5	N-Ethylbuphedrone	10
	3-Methylbuphedrone	35	3-Methylbuphedrone	7
	RCS-4 2-methoxy isomer	35	RCS-4 2-methoxy isomer	35
	Etaqualone	35	Etaqualone	31
	PB-22 6-hydroxyisoquinoline isomer	80	PB-22 6-hydroxyisoquinoline isomer*	110
	3-methyl- α -Pyrrolidinopropiophenone	80	3-methyl- α -Pyrrolidinopropiophenone	66
	Δ 8-THC	80	Δ 8-THC	90
	XLR11 N-(2-fluoropentyl) isomer	80	XLR11 N-(2-fluoropentyl) isomer	91
3	AB-FUBINACA	5	AB-FUBINACA*	7
	4-Methyl- α -ethylaminobutiophenone	5	4-Methyl- α -ethylaminobutiophenone	7
	THCA-A	5	nd	-
	MAM2201 N-pentanoic acid metabolite	5	MAM2201 N-pentanoic acid metabolite	6
	3C-B-fly	35	3C-B-fly	33
	3-methyl- α -Pyrrolidinopropiophenone	35	3-methyl- α -Pyrrolidinopropiophenone	33
	3,4-dimethoxy- α -Pyrrolidinopentiophenone	35	3,4-dimethoxy- α -Pyrrolidinopentiophenone	27

	N,N-dimethylcathinone	35	N,N-dimethylcathinone	42
	AM694 N-(5-hydroxypentyl) metabolite	80	AM694 N-(5-hydroxypentyl) metabolite	72
4	25I-NBF	5	25I-NBF	9
	4'-Methyl-N-methylhexanophenone	5	4'-Methyl-N-methylhexanophenone	8
	2C-T-2	35	2C-T-2	37
	4-hydroxy MET	80	4-hydroxy MET	64
5	4'-fluoro-a-Pyrrolidinopropiophenone	5	nd	-
	A-796260	5	A-796260	7
	N-Methyltryptamine	35	N-Methyltryptamine	40

*Isomer of this compound were also detected.

nd - not detected.

Finally, two sets of blind spiked whole blood samples were prepared and analyzed qualitatively and quantitatively. The first set was designed to test the lower limits of the validated method, while the second set was designed to be in the middle of the calibration curve. The experimental identity, experimental concentration, true identity, and true concentration for the 15 blind spikes included in the first set are shown in Table 25. All samples in the first set were extracted using an in-house mini one-pot QuEChERS approach. The only sample that was not properly identified was sample 1, which should have been identified as THCA-A, but was not. This was likely due to the fact that THCA-A was one of the few compounds that did not show linearity and therefore this method was not capable of properly identifying that compound. However, there were 3 samples that were determined to be blank even though they all contained one compound. Additionally, sample 5 contained two compounds but only one was identified. Even though the dMRM method used for analysis was validated specifically for urine, the whole blood spiked samples were also quantitated. Many of the compounds were identified as higher concentrations than the true value this could be due to matrix effects that differ from those seen with urine.

The second set was designed to be in the middle of the calibration curve. The experimental identity, experimental concentration, true identity, and true concentration for the 18 blind spikes included in the second set are shown in Table 26. All samples in the second set were extracted prepared using a crash-and-shoot approach. The only sample that was not properly identified was sample 1, which should have been identified as THCA-A, but was not. This was likely due to the fact that THCA-A was one of the few compounds that did not show linearity. Experimental concentrations for the majority

of compounds fell within ± 20 nominal showing that the method is capable of quantifying whole blood extracts with similar percent error to those seen with urine samples.

Table 25. The true identity and concentration and experimental identity and concentration of blind spiked whole blood samples included in set 1 (low concentration)

#	Compound Spiked	Conc. spiked (ng/mL)	Compound Detected	Conc. Detected (ng/mL)
1	THCA-A	5	JWH 200 5-hydroxyindole metabolite	N/A
2	4-APDB	5	-	-
3	JWH 200 5-hydroxyindole metabolite	5	JWH 200 5-hydroxyindole metabolite	9
4	N-Methyltryptamine	5	-	-
5	3-Methylbuphedrone	5	3-Methylbuphedrone	2
	5-fluoro SDB-006	5	-	-
6	2-fluoromethcathinone	5	-	-
7	2C-T-4	5	2C-T-4	3
8	PB-22	5	PB-22*	8
9	4-ethyl-N,N-dimethylcathinone	5	4-ethyl-N,N-dimethylcathinone	5
10	JWH 018 N(5-hydroxypentyl) metabolite	5	JWH 018 N(5-hydroxypentyl) metabolite	8
	MAM2201 N-pentanoic acid metabolite	5	MAM2201 N-pentanoic acid metabolite	5
11	25E-NBOMe	5	25E-NBOMe	4
12	AB-FUBINACA	5	AB-FUBINACA*	9
13	3C-B-fly	5	3C-B-fly	6
14	Para-Fluorofentanyl	5	Para-Fluorofentanyl	4
15	N,N-dimethylcathinone	5	N,N-dimethylcathinone	2
	Phenylpiperazine	5	Phenylpiperazine	4

*Isomer of this compound were also detected

Table 26. The true identity and concentration and experimental identity and concentration of blind spiked whole blood samples included in set 2 (medium concentration)

#	Compound Spiked into Urine	Conc. spiked (ng/mL)	Compound Detected	Conc. Detected (ng/mL)
1	THCA-A	50	Benzydamine	N/A
2	4-APDB	50	4-APDB	98
3	JWH 200 5-hydroxyindole metabolite	50	JWH 200 5-hydroxyindole metabolite	34
4	blank	0	None	-
5	N-Methyltryptamine	50	N-Methyltryptamine	46
6	3-Methylbuphedrone	50	3-Methylbuphedrone	8
	5-fluoro SDB-006	50	5-fluoro SDB-006	24
7	2-fluoromethcathinone	50	2-fluoromethcathinone	47
8	2C-T-4	50	2C-T-4	55
9	PB-22	50	PB-22*	66
10	blank	0	None	-
11	4-ethyl-N,N-dimethylcathinone	50	4-ethyl-N,N-dimethylcathinone	12
12	JWH 018 N(5-hydroxypentyl) metabolite	50	JWH 018 N(5-hydroxypentyl) metabolite*	36
	MAM2201 N-pentanoic acid metabolite	50	MAM2201 N-pentanoic acid metabolite	26
13	25E-NBOMe	50	25E-NBOMe	40
14	AB-FUBINACA	50	AB-FUBINACA*	43
15	3C-B-fly	50	3C-B-fly	37
16	para-Fluorofentanyl	50	para-Fluorofentanyl	23
17	blank	0	None	-
18	N,N-dimethylcathinone	50	N,N-dimethylcathinone	6
	Phenylpiperazine	50	Phenylpiperazine	41

*Isomer of this compound were also detected

In a final test of applicability, a total of 50 authentic urine specimens were qualitatively analyzed using a method with transitions for all of the 16 standard mixes, therefore allowing screening for any of the 729 compounds included. Table 27 shows the identity of the compounds found and the number of specimens that were positive for each compound, while Table 28 shows the identity of metabolites found following glucuronidase treatment and the number of specimens positive for each. A general toxicology screen looking for common drugs of abuse and their metabolites was completed in addition to the NPS screen. The results of the general screening are shown in Table 29. The results of the NPS screen and general screening for each of the 50 authentic specimens can be seen in Table 30. Cathine and levamisole were present in the highest number of specimens. Cathine is a metabolite of pseudophedrine and likely represents use of this common over the counter (OTC) drug rather than direct ingestion of cathine.¹¹⁹ Levamisole is a common adulterant of cocaine, which explains why it is present in the majority of samples that were positive for beanzoylecgonine. Interestingly, several NPS/metabolites were also confirmed present in at least one specimen, illustrating the potential value of this method for identification of compounds not typically screened for in forensic specimens.

Table 27. The identity of compounds found in authentic specimens and the number of positive specimens for each compound.

Compound Detected	Number of Positives
Levamisole	12
Cathine	11
6-IT	5
2C-I	4
4-methoxy-N,N-dimethylcathinone	4
Hydroxy Bupropion	4
Sildenafil Citrate	4
3-Bromomethcathinone	3
4-FMC	3
3-fluoromethcathinone	2
3-methoxyamphetamine	2
6-APB	2
Mescaline	2
2,3-Dichlorophenylpiperazine	1
4-acetoxy DMT	1
4-hydroxy MET	1
4-methyl- α -ethylaminobutiophenone	1
Benzylamine	1
Deoxypipradol	1
Loperamide	1
Propylhexedrine	1

Table 28. The identity of metabolites found in authentic specimens following glucuronidase treatment and the number of positive specimens for each compound.

Compound Detected	Number of Positives
4-fluoromethcathinone metabolite	10
Buphedrone metabolite	5
Pentedrone metabolite	4
JWH 200 7-hydroxyindole metabolite	3
JWH 073 5-hydroxyindole metabolite	1

Table 29. The identity of compounds found in authentic specimens after a general screening and the number of positive specimens for each compound.

Compound Detected	Number of Positives
Pregabalin	46
Morphine	19
Buprenorphine	17
Norbuprenorphine	16
Gabapentin	14
Ritalinic Acid	10
Dextroprhan	9
Oxazepam	8
Oxycodone	8
Alprazolam	7
Beonzoylcegonine (cocaine)	6
OH-Alprazolam	6
Oxymorphone	6
Temazepam	6
Doxepin	5
Nordiazepam	5
Ethylmorphine	4
Hydrocodone	4
Amphetamine	3
Hydromorphone	3
M6G	3
Methamphetamine	3
Norephedrine	3
Dextromethorphan	2
EDDP	2
Methadone	2
C6G	1
Codeine	1
Lorazepam	1
MDMA	1
Mitragynine	1
OH-Mitragynine	1
PMMA	1
THC	1
Tramadol	1

Table 30. The identity of compounds found in each individual authentic specimen during the general screening and the NPS screening

Specimen #	Compounds Detected in NPS Screen	Compounds Detected in Routine Screen
513	cathine, levamisole	beonzoylcegonine, gabapentin, MDMA, norephedrine, pregabalin, ritalinic acid, tramadol
514	cathine, levamisole, 3-fluoromethcathinone, 4-FMC, 4-methoxy-N,N-dimethylcathinone	beonzoylcegonine, buprenorphine, hydrocodone, oxycodone, oxymorphone, pregabalin
515	cathine	alprazolam, amphetamine, methamphetamine, norephedrine, oh-alprazolam, pregabalin, THC
516	3-methoxyamphetamine	hydrocodone, pregabalin
517	3-methoxyamphetamine	alprazolam, doxepin, EDDP, methadone, oxazepam, pregabalin, temazepam
518	levamisole, hydroxy bupropion	doxepin, pregabalin
519	levamisole, 4-acetoxy DMT	beonzoylcegonine, buprenorphine, doxepin, morphine, norbuprenorphine, pregabalin
520	4-hydroxy MET, 4-methoxy-N,N-dimethylcathinone	doxepin, morphine, oxazepam, pregabalin
521	hydroxy bupropion, propylhexedrine	pregabalin
522	2,3-dichlorophenylpiperazine, deoxypipradol, loperamide	morphine, pregabalin
523	sildenafil	morphine, pregabalin
524	nd	buprenorphine, gabapentin, norbuprenorphine, oxymorphone, pregabalin, ritalinic acid
525	sildenafil	pregabalin, ritalinic acid
526	sildenafil	buprenorphine, gabapentin, norbuprenorphine, pregabalin
527	mescaline	alprazolam, EDDP, methadone, oh-alprazolam, pregabalin
528	nd	pregabalin
529	mescaline,	ethylmorphine, gabapentin, pregabalin
530	nd	pregabalin
531	cathine, levamisole	pregabalin
532	nd	pregabalin
533	sildenafil	morphine, pregabalin, ritalinic acid

534	cathine, 4-FMC	buprenorphine, dextromethorphan, dextroprhan, gabapentin, norbuprenorphine
535	nd	C6G, codeine, dextroprhan, M6G, mitragynine, morphine, oh-mitragynine, pregabalin
536	3-fluoromethcathinone	buprenorphine, dextroprhan, morphine, norbuprenorphine, pregabalin, ritalinic acid
537	nd	buprenorphine, dextroprhan, norbuprenorphine, nordiazepam, oxazepam, pregabalin, temazepam
538	nd	dextroprhan, pregabalin
539	3-bromomethcathinone, cathine	amphetamine, buprenorphine, dextroprhan, lorazepam, morphine, norbuprenorphine, nordiazepam, norephedrine, oxazepam, PMMA, pregabalin, temazepam
540	hydroxy bupropion	ethylmorphine, pregabalin
541	4-methoxy-N,N-dimethylcathinone, 6-IT	amphetamine, pregabalin
542	cathine, 4-FMC	alprazolam, buprenorphine, dextroprhan, hydrocodone, norbuprenorphine, nordiazepam, oh-alprazolam, oxazepam, oxycodone, oxymorphone, pregabalin, temazepam
543	6-IT	buprenorphine, dextroprhan, ethylmorphine, gabapentin, morphine, pregabalin, ritalinic acid
544	levamisole, benzydamine	alprazolam, buprenorphine, ethylmorphine, hydromorphone, M6G, morphine, norbuprenorphine, nordiazepam, oh-alprazolam, oxazepam, oxymorphone, pregabalin, temazepam
545	cathine	alprazolam, buprenorphine, morphine, norbuprenorphine, oh-alprazolam, pregabalin
546	nd	pregabalin
547	levamisole, 6-IT	beonzylecgonine, buprenorphine, dextromethorphan, dextroprhan, gabapentin, methamphetamine, morphine, norbuprenorphine
548	levamisole, 4-methoxy-N,N-dimethylcathinone	buprenorphine, doxepin, morphine, norbuprenorphine, pregabalin
549	cathine, levamisole	pregabalin, ritalinic acid
550	nd	hydrocodone, morphine, pregabalin
551	4-methyl- α -ethylaminobutiophenone	pregabalin

552	levamisole	beonzoylecgonine, buprenorphine, morphine, norbuprenorphine, pregabalin
553	3-bromomethcathinone	buprenorphine, gabapentin, morphine, norbuprenorphine, nordiazepam, oxazepam, PMMA, pregabalin, temazepam
554	cathine	alprazolam, methamphetamine, oh-alprazolam, pregabalin
555	cathine	pregabalin
556	hydroxy bupropion	nd
557	nd	beonzoylecgonine, buprenorphine, gabapentin, hydromorphone, morphine, norbuprenorphine, oxycodone, oxymorphone, pregabalin, ritalinic acid
558	nd	gabapentin, hydromorphone, M6G, morphine, oxymorphone, ritalinic acid
559	levamisole	gabapentin, morphine, oxazepam, pregabalin, ritalinic acid
560	levamisole	gabapentin, pregabalin
596	3-bromomethcathinone	gabapentin, pregabalin
597	nd	gabapentin, pregabalin

nd - no detections

6.3 Conclusions

In order to further test the applicability of the validated dMRM method blind spiked samples in urine and whole blood and authentic urine specimens analyzed. Testing blind spikes and authentic specimens made it possible to determine the method's potential as both a screening and confirmatory method. The results from screening and confirmation of the blind spiked samples it was shown that the dMRM method has potential to be used as a confirmatory method for samples in urine and a screening method for samples in whole blood. Due to the number of NPS included in this method it is a semi-qualitative method because it is impossible to match every compound with a deuterated internal standard. Since some compounds do not have an ideal match when quantitated the compound may undergo different matrix effects and ionization than the paired internal standard. However, the results from the blind spike studies and authentic specimens with the validated method indicate that it shows great potential as a comprehensive screening method for the the largest number of NPS reported to date.

7. SUMMARY AND PROSPECT

Novel psychoactive substances (NPS) have gained popularity over the past two decades all over the world and it does not seem that there will be an end soon. The increased popularity of NPS makes it imperative that clinical and forensic toxicological laboratories have access to reliable comprehensive screening methods for NPS. Unlike with common drugs of abuse, immunoassays are not capable of selectively detecting NPS due to their multiple structural alterations. Immunoassays are one of the most common screening methods for clinical and forensic human specimens. However, they need to be replaced by methods capable of reliably screening for a large number of NPS within varying drug classes. Alternative screening methods do exist, some of which are capable of detecting NPS. Instrumental screening methods (*i.e.*, GC-MS and LC-MS) can be used to screen clinical and forensic toxicology specimens. These methods typically work based on spectral library or database matches. It is vital that spectral libraries and databases exist that include NPS in order to properly screen for them in clinical and forensic toxicological samples.

The goal of the research presented here was to aid in the screening and confirmation of NPS in clinical and forensic toxicological specimens. An MRM transition ion database and a comprehensive screening and confirmatory dMRM method for the detection of NPS in biological matrices were created and are the largest of their kind. In addition to the creation of a dMRM method, validation using a mixture approach was completed to ensure that method parameters fell within OSAC guidelines. Often, method validation is done

using small mixtures or a one-at-a-time approach, however, this was not feasible for the quantity of NPS that this method was designed to screen for. Consequently, an approach using a series of mixtures of non-coeluting NPS standards was adapted in order to greatly reduce the time it takes to fully validate a comprehensive screening method. Blind spiked urine samples were screened and quantitated using the described dMRM method. This was done to further validate the selectivity and sensitivity of the method as it would be used in the field of forensics. The majority of NPS were correctly identified and most concentrations were determined to be within $\pm 20\%$ of the spiked concentration showing the selectivity and sensitivity of the overall method. Additionally, the dMRM method was used to screen 50 authentic urine specimens to show real world relevance of the method. From the 50 specimens 21 compounds were detected including NPS (synthetic cathinones) showing the potential of this method for clinical and forensic toxicological specimens.

It is not uncommon that biological matrices must undergo extraction and/or purification before they can be injected into an instrument and analyzed. Consequently, this project also aimed to evaluate extraction techniques for NPS in whole blood and urine. Depending on the complexity of the matrix, developing and optimizing an extraction technique can be very difficult. The research described here was designed to determine if particular extraction methods were more efficient than others for the extraction of NPS from whole blood and urine. Forensic toxicological laboratories in general have standard extraction procedures in place for common drugs of abuse, typically involving protein precipitation and/or SPE. The procedure of protein precipitation does not differ from one compound to the next, however SPE involves complex chemistry and relies on pH and pKa

of the compounds in the sample. Therefore, SPE and potentially other extraction techniques need to be optimized for NPS. This research delved into the usefulness of dilute-and-shoot, crash-and-shoot, online-SPE, classical SPE, and QuEChERS for the extraction of NPS from biological matrices.

This work investigated the potential of on-line SPE and QuEChERS as alternative extraction technique for NPS, since neither technique has been commonly used in forensic toxicology laboratories. QuEChERS is an appealing technique for complex matrices and is more time and cost efficient than SPE. On-line SPE is much more time efficient but was found to involve extremely complex method development. QuEChERS is an appealing alternative to traditional extraction techniques, since it can easily be implemented into different clinical and forensic toxicological laboratories with simple purchase of reagents but not requiring additional instrumentation. The one disadvantage of QuEChERS as compared to classical SPE is the elimination of matrix effects. This is an important factor to consider when determining the needs of an extraction technique.

Future work will be necessary in order to update and expand upon the database and dMRM method as more NPS are reported in literature, however, this will require the availability of appropriate reference standards. Further optimization of QuEChERS would be needed to increase the elimination of matrix effects with the goal of having comparable results to that of SPE. When considering the time and cost efficiency of QuEChERS, it would be a beneficial extraction technique to be implemented into forensic toxicology laboratories. Since QuEChERS is designed for complex matrices it has even further

potential to be used to extract NPS from biological matrices other than urine and whole blood.

REFERENCES

1. Elliott, S.; Evans, J. A 3-year review of new psychoactive substances in casework. *Forensic Science International* **2014**, *243*, 55-60.
2. Liechti, M. E. Novel psychoactive substances (designer drugs): overview and pharmacology of modulators of monoamine signalling. *Swiss Medical Weekly* **2015**, *145*, w14043.
3. Manchester, K. R.; Lomas, E. C.; Waters, L.; Dempsey, F. C.; Maskell, P. D. The emergence of new psychoactive substance (NPS) benzodiazepines: A review. *Drug Testing and Analysis* **2018**, *10* (1), 37-53.
4. Palamar, J. J.; Le, A. Use of new and uncommon synthetic psychoactive drugs among a nationally representative sample in the United States, 2005-2017. *Human Psychopharmacology: Clinical and Experimental* **2019**, e2690.
5. Coulson, C.; Caulkins, J. P. Scheduling of newly emerging drugs: A critical review of decisions over 40 years. *Addiction* **2012**, *107* (4), 766-773.
6. Dargan, P.; Wood, D. Novel psychoactive substances: classification, pharmacology and toxicology; *Academic Press*: 2013.
7. Zawilska, J. B.; Andrzejczak, D. Next generation of novel psychoactive substances on the horizon: A complex problem to face. *Drug and Alcohol Dependence* **2015**, *157*, 1-17.
8. Montesano, C.; Vannutelli, G.; Gregori, A.; Ripani, L.; Compagnone, D.; Curini, R.; Sergi, M. Broad screening and identification of novel psychoactive substances in plasma by high-performance liquid chromatography high-resolution mass spectrometry and post-run library matching. *Journal of Analytical Toxicology* **2016**, *40* (7), 519-528.
9. Peters, F. T. Recent advances of liquid chromatography (tandem) mass spectrometry in clinical and forensic toxicology. *Clinical Biochemistry* **2011**, *44* (1), 54-65.

10. Reuter, P.; Pardo, B. New psychoactive substances: Are there any good options for regulating new psychoactive substances? *International Journal of Drug Policy* **2017**, *40*, 117-122.
11. Favretto, D.; Pascali, J. P.; Tagliaro, F. New challenges and innovation in forensic toxicology: focus on the "New Psychoactive Substances". *Journal of Chromatography A* **2013**, *1287*, 84-95.
12. Sun, H. q.; Bao, Y. p.; Zhou, S. j.; Meng, S. q.; Lu, L. The new pattern of drug abuse in China. *Current Opinion in Psychiatry* **2014**, *27* (4), 251-255.
13. Zaami, S.; Giorgetti, R.; Pichini, S.; Pantano, F.; Marinelli, E.; Busardo, F. P. Synthetic cathinones related fatalities: an update. *European Review of Medicine and Pharmacological Science* **2018**, *22* (1), 268-274.
14. Palamar, J. J.; Martins, S. S.; Su, M. K.; Ompad, D. C. Self-reported use of novel psychoactive substances in a US nationally representative survey: Prevalence, correlates, and a call for new survey methods to prevent underreporting. *Drug and Alcohol Dependence* **2015**, *156*, 112-119.
15. Title 21 United States Code (U.S.C.) . Controlled Substances Act. 2018. U.S.Department of Justice, Drug Eforcement Administration Division Control Division.
16. Tang, M. H. Y.; Ching, C. K.; Tsui, M. S. H.; Chu, F. K. C.; Mak, T. W. L. Two cases of severe intoxication associated with analytically confirmed use of the novel psychoactive substances 25B-NBOMe and 25C-NBOMe. *Clinical Toxicology* **2014**, *52* (5), 561-565.
17. Abbate, V.; Schwenk, M.; Presley, B. C.; Uchiyama, N. The ongoing challenge of novel psychoactive drugs of abuse. Part I. Synthetic cannabinoids (IUPAC Technical Report). *Pure and Applied Chemistry* **2018**, *90* (8), 1255-1282.
18. Wiley, J. L.; Marusich, J. A.; Huffman, J. W.; Balster, R. L.; Thomas, B. F. Hijacking of basic research: the case of synthetic cannabinoids. *Methods Report (RTI Press)* **2011**, *2011*.
19. Tittarelli, R.; Mannocchi, G.; Pantano, F.; Saverio Romolo, F. Recreational use, analysis and toxicity of tryptamines. *Current Neuropharmacology* **2015**, *13* (1), 26-46.
20. Koch, K.; Auwärter, V.; Hermanns-Clausen, M.; Wilde, M.; Neukamm, M. A. Mixed intoxication by the synthetic opioid U47700 and the benzodiazepine flubromazepam with lethal outcome: Pharmacokinetic data. *Drug Testing and Analysis* **2018**, *10* (8), 1336-1341.

21. Nikolaou, P.; Papoutsis, I.; Stefanidou, M.; Spiliopoulou, C.; Athanaselis, S. 2C-I-NBOMe, an "N-bomb" that kills with "Smiles" toxicological and legislative aspects. *Drug and Chemical Toxicology* **2015**, 38 (1), 113-119.
22. Shanks, K. G.; Sozio, T.; Behonick, G. S. Fatal intoxications with 25B-NBOMe and 25I-NBOMe in Indiana during 2014. *Journal of Analytical Toxicology* **2015**, 39 (8), 602-606.
23. H-iseth, G.; Tuv, S. S.; Karinen, R. Blood concentrations of new designer benzodiazepines in forensic cases. *Forensic Science International* **2016**, 268, 35-38.
24. Prosser, J. M.; Nelson, L. S. The toxicology of bath salts: a review of synthetic cathinones. *Journal of Medical Toxicology* **2012**, 8 (1), 33-42.
25. Seely, K. A.; Patton, A. L.; Moran, C. L.; Womack, M. L.; Prather, P. L.; Fantegrossi, W. E.; Radomska-Pandya, A.; Endres, G. W.; Channell, K. B.; Smith, N. H. Forensic investigation of K2, Spice, and "bath salt" commercial preparations: a three-year study of new designer drug products containing synthetic cannabinoid, stimulant, and hallucinogenic compounds. *Forensic Science International* **2013**, 233 (1-3), 416-422.
26. Smith, J. P.; Sutcliffe, O. B.; Banks, C. E. An overview of recent developments in the analytical detection of new psychoactive substances (NPSs). *Analyst* **2015**, 140 (15), 4932-4948.
27. Couto, R. A.; Goncalves, L. s. M.; Carvalho, F.; Rodrigues, J. A.; Rodrigues, C. I. M.; Quinaz, M. B. The analytical challenge in the determination of cathinones, key-players in the worldwide phenomenon of novel psychoactive substances. *Critical Reviews in Analytical Chemistry* **2018**, 1-19.
28. Spiller, H. A.; Ryan, M. L.; Weston, R. G.; Jansen, J. Clinical experience with and analytical confirmation of "bath salts" and "legal highs"(synthetic cathinones) in the United States. *Clinical Toxicology* **2011**, 49 (6), 499-505.
29. Hasegawa, K.; Wurita, A.; Minakata, K.; Gonmori, K.; Nozawa, H.; Yamagishi, I.; Suzuki, O.; Watanabe, K. Identification and quantitation of a new cathinone designer drug PV9 in an aroma liquid product, antemortem whole blood and urine specimens, and a postmortem whole blood specimen in a fatal poisoning case. *Forensic Toxicology* **2014**, 32 (2), 243-250.

30. Ellefsen, K. N.; Concheiro, M.; Huestis, M. A. Synthetic cathinone pharmacokinetics, analytical methods, and toxicological findings from human performance and postmortem cases. *Drug Metabolism Reviews* **2016**, *48* (2), 237-265.
31. Weinstein, A. M.; Rosca, P.; Fattore, L.; London, E. D. Synthetic cathinone and cannabinoid designer drugs pose a major risk for public health. *Frontiers in Psychiatry* **2017**, *8*, 156.
32. Ashrafioun, L.; Bonadio, F. A.; Baik, K. D.; Bradbury, S. L.; Carhart, V. L.; Cross, N. A.; Davis, A. K.; Feuille, M.; Harper, A. R.; Lackey, J. H. Patterns of use, acute subjective experiences, and motivations for using synthetic cathinones ("bath salts") in recreational users. *Journal of Psychoactive Drugs* **2016**, *48* (5), 336-343.
33. Morad, A. M.; Al-Meshal, I. A.; Nasir, M.; El-Feraly, F. S. High-performance liquid chromatographic determination of cathinone in plasma. *Chromatographia* **1989**, *27* (5-6), 201-204.
34. Johnson, R. D.; Botch-Jones, S. R. The stability of four designer drugs: MDPV, mephedrone, BZP and TFMPP in three biological matrices under various storage conditions. *Journal of Analytical Toxicology* **2013**, *37* (2), 51-55.
35. Kerrigan, S.; Savage, M.; Cavazos, C.; Bella, P. Thermal degradation of synthetic cathinones: implications for forensic toxicology. *Journal of Analytical Toxicology* **2016**, *40* (1), 1-11.
36. Radomska-Pandya, A.; Moran, J.; Fantegrossi, W.; Prather, P. Synthetic cannabinoids: Rapidly emerging drugs of abuse. *The FASEB Journal* **2017**, *31* (1_supplement), 811.
37. Centers for Disease Control and Prevention . Synthetic cannabinoids: What are they? What are their effects? 4-24-2018. 4-14-2019.
38. Huffman, J. W.; Yu, S.; Showalter, V.; Abood, M. E.; Wiley, J. L.; Compton, D. R.; Martin, B. R.; Bramblett, R. D.; Reggio, P. H. Synthesis and pharmacology of a very potent cannabinoid lacking a phenolic hydroxyl with high affinity for the CB2 receptor. *Journal of Medicinal Chemistry* **1996**, *39* (20), 3875-3877.
39. Debruyne, D. I.; Le Boisselier, R. Emerging drugs of abuse: current perspectives on synthetic cannabinoids. *Substance Abuse and Rehabilitation* **2015**, *6*, 113.

40. Tait, R. J.; Caldicott, D.; Mountain, D.; Hill, S. L.; Lenton, S. A systematic review of adverse events arising from the use of synthetic cannabinoids and their associated treatment. *Clinical Toxicology* **2016**, *54* (1), 1-13.
41. Adams, A. J.; Banister, S. D.; Irizarry, L.; Trecki, J.; Schwartz, M.; Gerona, R. "Zombie" outbreak caused by the synthetic cannabinoid AMB-FUBINACA in New York. *New England Journal of Medicine* **2017**, *376* (3), 235-242.
42. Knittel, J. L.; Holler, J. M.; Chmiel, J. D.; Vorce, S. P.; Magluilo Jr, J.; Levine, B.; Ramos, G.; Bosy, T. Z. Analysis of parent synthetic cannabinoids in blood and urinary metabolites by liquid chromatography tandem mass spectrometry. *Journal of Analytical Toxicology* **2016**, *40* (3), 173-186.
43. Armenian, P.; Vo, K. T.; Barr-Walker, J.; Lynch, K. L. Fentanyl, fentanyl analogs and novel synthetic opioids: a comprehensive review. *Neuropharmacology* **2018**, *134*, 121-132.
44. Jalal, H.; Buchanich, J. M.; Roberts, M. S.; Balmert, L. C.; Zhang, K.; Burke, D. S. Changing dynamics of the drug overdose epidemic in the United States from 1979 through 2016. *Science* **2018**, *361* (6408), eaau1184.
45. Jannetto, P. J.; Helander, A.; Garg, U.; Janis, G. C.; Goldberger, B.; Ketha, H. The Fentanyl epidemic and evolution of fentanyl analogs in the United States and the European Union. *Clinical Chemistry* **2019**, *65* (2), 242-253.
46. O'Donnell, J. K.; Halpin, J.; Mattson, C. L.; Goldberger, B. A.; Gladden, R. M. Deaths involving fentanyl, fentanyl analogs, and U-47700: 10 states, July-December 2016. *MMWR. Morbidity and Mortality Weekly Report* **2017**, *66* (43), 1197.
47. Lozier, M. J.; Boyd, M.; Stanley, C.; Ogilvie, L.; King, E.; Martin, C.; Lewis, L. Acetyl fentanyl, a novel fentanyl analog, causes 14 overdose deaths in Rhode Island, March-May 2013. *Journal of Medical Toxicology* **2015**, *11* (2), 208-217.
48. Rogers, J. S.; Rehrer, S. J.; Hoot, N. R. Acetylfentanyl: an emerging drug of abuse. *The Journal of Emergency Medicine* **2016**, *50* (3), 433-436.
49. Ostrea Jr, E. M.; Brady, M. J.; Parks, P. M.; Asensio, D. C.; Naluz, A. Drug screening of meconium in infants of drug-dependent mothers: an alternative to urine testing. *The Journal of Pediatrics* **1989**, *115* (3), 474-477.

50. Strano-Rossi, S. A. B. I.; Bermejo-Barrera, A.; Chiarotti, M. Segmental hair analysis for cocaine and heroin abuse determination. *Forensic Science International* **1995**, *70* (1-3), 211-216.
51. Cone, E. J. Legal, workplace, and treatment drug testing with alternate biological matrices on a global scale. *Forensic Science International* **2001**, *121* (1-2), 7-15.
52. Maurer, H. H. Current role of liquid chromatography mass spectrometry in clinical and forensic toxicology. *Analytical and Bioanalytical Chemistry* **2007**, *388* (7), 1315-1325.
53. Shima, N.; Katagi, M.; Kamata, H.; Matsuta, S.; Sasaki, K.; Kamata, T.; Nishioka, H.; Miki, A.; Tatsuno, M.; Zaitso, K. Metabolism of the newly encountered designer drug +-pyrrolidinovalerophenone in humans: identification and quantitation of urinary metabolites. *Forensic Toxicology* **2014**, *32* (1), 59-67.
54. Moeller, M. R.; Steinmeyer, S.; Kraemer, T. Determination of drugs of abuse in blood. *Journal of Chromatography B: Biomedical Sciences and Applications* **1998**, *713* (1), 91-109.
55. Levine, B. Principle of Forensic Toxicology. **2019**.
56. Bertol, E.; Vaiano, F.; Borsotti, M.; Quercioli, M.; Mari, F. Comparison of immunoassay screening tests and LC MS-MS for urine detection of benzodiazepines and their metabolites: results of a national proficiency test. *Journal of Analytical Toxicology* **2013**, *37* (9), 659-664.
57. Nakanishi, K.; Miki, A.; Zaitso, K.; Kamata, H.; Shima, N.; Kamata, T.; Katagi, M.; Tatsuno, M.; Tsuchihashi, H.; Suzuki, K. Cross-reactivities of various phenethylamine-type designer drugs to immunoassays for amphetamines, with special attention to the evaluation of the one-step urine drug test Instant-View, and the Emit assays for use in drug enforcement. *Forensic Science International* **2012**, *217* (1-3), 174-181.
58. Nieddu, M.; Trignano, C.; Burrai, L.; Pirisi, M. A.; Boatto, G. Cross-reactivities of 41 new amphetamine designer drugs to EMIT immunoassays. *Forensic Toxicology* **2013**, *31* (1), 133-137.
59. Beck, O.; Rausberg, L.; AlGhÉSaffar, Y.; Villen, T.; Karlsson, L.; Hansson, T.; Helander, A. Detectability of new psychoactive substances, "legal highs", in CEDIA, EMIT, and KIMS immunochemical screening assays for drugs of abuse. *Drug Testing and Analysis* **2014**, *6* (5), 492-499.

60. Swortwood, M. J.; Hearn, W. L.; DeCaprio, A. P. Cross-reactivity of designer drugs, including cathinone derivatives, in commercial enzyme-linked immunosorbent assays. *Drug Testing and Analysis* **2014**, 6 (7-8), 716-727.
61. Arntson, A.; Ofsa, B.; Lancaster, D.; Simon, J. R.; McMullin, M.; Logan, B. Validation of a novel immunoassay for the detection of synthetic cannabinoids and metabolites in urine specimens. *Journal of Analytical Toxicology* **2013**, 37 (5), 284-290.
62. Kerrigan, S.; Mellon, M. B.; Banuelos, S.; Arndt, C. Evaluation of commercial enzyme-linked immunosorbent assays to identify psychedelic phenethylamines. *Journal of Analytical Toxicology* **2011**, 35 (7), 444-451.
63. Wang, G.; Huynh, K.; Barhate, R.; Rodrigues, W.; Moore, C.; Coulter, C.; Vincent, M.; Soares, J. Development of a homogeneous immunoassay for the detection of fentanyl in urine. *Forensic Science International* **2011**, 206 (1-3), 127-131.
64. Ellefsen, K. N.; Anizan, S.; Castaneto, M. S.; Desrosiers, N. A.; Martin, T. M.; Klette, K. L.; Huestis, M. A. Validation of the only commercially available immunoassay for synthetic cathinones in urine: Randox Drugs of Abuse V Biochip Array Technology. *Drug Testing and Analysis* **2014**, 6 (7-8), 728-738.
65. de Hoffmann, E.; Stroobant, V. *Mass spectrometry: principles and applications*; 2007.
66. Remane, D.; Wissenbach, D. K.; Peters, F. T. Recent advances of liquid chromatography (tandem) mass spectrometry in clinical and forensic toxicology: an update. *Clinical Biochemistry* **2016**, 49 (13-14), 1051-1071.
67. Peters, F. T.; Remane, D. Aspects of matrix effects in applications of liquid chromatography mass spectrometry to forensic and clinical toxicology: a review. *Analytical and Bioanalytical Chemistry* **2012**, 403 (8), 2155-2172.
68. Banerjee, S.; Mazumdar, S. Electrospray ionization mass spectrometry: a technique to access the information beyond the molecular weight of the analyte. *International Journal of Analytical Chemistry* **2012**, 2012.
69. Oberacher, H.; Arnhard, K. Current status of non-targeted liquid chromatography-tandem mass spectrometry in forensic toxicology. *TrAC Trends in Analytical Chemistry* **2016**, 84, 94-105.

70. Oberacher, H. Applying tandem mass spectral libraries for solving the critical assessment of small molecule identification (CASMI) LC/MS challenge 2012. *Metabolites* **2013**, 3 (2), 312-324.
71. Dresen, S.; Kempf, J.; Weinmann, W. Electrospray-ionization MS/MS library of drugs as database for method development and drug identification. *Forensic Science International* **2006**, 161 (2-3), 86-91.
72. Dresen, S.; Gergov, M.; Politi, L.; Halter, C.; Weinmann, W. ESI-MS/MS library of 1,253 compounds for application in forensic and clinical toxicology. *Analytical and Bioanalytical Chemistry* **2009**, 395 (8), 2521.
73. Causanilles, A.; Kinyua, J.; Ruttkies, C.; van Nuijs, A. L. N.; Emke, E.; Covaci, A.; de Voogt, P. Qualitative screening for new psychoactive substances in wastewater collected during a city festival using liquid chromatography coupled to high-resolution mass spectrometry. *Chemosphere* **2017**, 184, 1186-1193.
74. Meyer, M. R.; Maurer, H. H. LC coupled to low-and high-resolution mass spectrometry for new psychoactive substance screening in biological matrices: where do we stand today? *Analytica Chimica Acta* **2016**, 927, 13-20.
75. Seither, J. Z.; Hindle, R.; Arroyo-Mora, L. E.; DeCaprio, A. P. Systematic analysis of novel psychoactive substances. I. Development of a compound database and HRMS spectral library. *Forensic Chemistry* **2018**, 9, 12-20.
76. Ojanperä, I.; Kolmonen, M.; Pelander, A. Current use of high-resolution mass spectrometry in drug screening relevant to clinical and forensic toxicology and doping control. *Analytical and Bioanalytical Chemistry* **2012**, 403 (5), 1203-1220.
77. Pasin, D.; Cawley, A.; Bidny, S.; Fu, S. Current applications of high-resolution mass spectrometry for the analysis of new psychoactive substances: a critical review. *Analytical and Bioanalytical Chemistry* **2017**, 409 (25), 5821-5836.
78. Wohlfarth, A.; Weinmann, W.; Dresen, S. LC-MS/MS screening method for designer amphetamines, tryptamines, and piperazines in serum. *Analytical and Bioanalytical Chemistry* **2010**, 396 (7), 2403-2414.
79. Ammann, J.; McLaren, J. M.; Gerostamoulos, D.; Beyer, J. Detection and quantification of new designer drugs in human blood: part 1: Synthetic cannabinoids. *Journal of Analytical Toxicology* **2012**, 36 (6), 372-380.

80. Adamowicz, P.; Tokarczyk, B. Simple and rapid screening procedure for 143 new psychoactive substances by liquid chromatography-tandem mass spectrometry. *Drug Testing and Analysis* **2016**, 8 (7), 652-667.
81. Glicksberg, L.; Bryand, K.; Kerrigan, S. Identification and quantification of synthetic cathinones in blood and urine using liquid chromatography-quadrupole/time of flight (LC-Q/TOF) mass spectrometry. *Journal of Chromatography B* **2016**, 1035, 91-103.
82. Swortwood, M. J.; Boland, D. M.; DeCaprio, A. P. Determination of 32 cathinone derivatives and other designer drugs in serum by comprehensive LC-QQQ-MS/MS analysis. *Analytical and Bioanalytical Chemistry* **2013**, 405 (4), 1383-1397.
83. Wohlfarth, A.; Scheidweiler, K. B.; Chen, X.; Liu, H. f.; Huestis, M. A. Qualitative confirmation of 9 synthetic cannabinoids and 20 metabolites in human urine using LC-MS/MS and library search. *Analytical Chemistry* **2013**, 85 (7), 3730-3738.
84. Partridge, E.; Trobbiani, S.; Stockham, P.; Scott, T.; Kostakis, C. A Validated Method for the Screening of 320 Forensically Significant Compounds in Blood by LC/QTOF, with Simultaneous Quantification of Selected Compounds. *Journal of Analytical Toxicology* **2018**.
85. Vaiano, F.; Busardo, F. P.; Palumbo, D.; Kyriakou, C.; Fioravanti, A.; Catalani, V.; Mari, F.; Bertol, E. A novel screening method for 64 new psychoactive substances and 5 amphetamines in blood by LC-MS/MS and application to real cases. *Journal of Pharmaceutical and Biomedical Analysis* **2016**, 129, 441-449.
86. Dziadosz, M.; Teske, J.; Henning, K.; Klintschar, M.; Nordmeier, F. LC-MS/MS screening strategy for cannabinoids, opiates, amphetamines, cocaine, benzodiazepines and methadone in human serum, urine and post-mortem blood as an effective alternative to immunoassay based methods applied in forensic toxicology for preliminary examination. *Forensic Chemistry* **2018**, 7, 33-37.
87. Annesley, T. M. Ion suppression in mass spectrometry. *Clinical Chemistry* **2003**, 49 (7), 1041-1044.
88. Matuszewski, B. K.; Constanzer, M. L.; Chavez-Eng, C. M. Strategies for the assessment of matrix effect in quantitative bioanalytical methods based on HPLC MS/MS. *Analytical Chemistry* **2003**, 75 (13), 3019-3030.

89. Axel, M.; Ewelina, K.; Jenny-Maria, B.; Leif, K. An online SPE LC-MS/MS method for the analysis of antibiotics in environmental water. *Environmental Science and Pollution Research* **2017**, *24* (9), 8692-8699.
90. Heuett, N. V.; Ramirez, C. E.; Fernandez, A.; Gardinali, P. R. Analysis of drugs of abuse by online SPE-LC high resolution mass spectrometry: communal assessment of consumption. *Science of the Total Environment* **2015**, *511*, 319-330.
91. Moosavi, S. M.; Shekar, K.; Fraser, J.; Smith, M. T.; Ghassabian, S. High-throughput assay for quantification of the plasma concentrations of thiopental using automated solid phase extraction (SPE) directly coupled to LC-MS/MS instrumentation. *Journal of Chromatography B* **2016**, *1038*, 80-87.
92. Lehmann, S.; Kieliba, T.; Beike, J.; Thevis, M.; Mercer-Chalmers-Bender, K. Determination of 74 new psychoactive substances in serum using automated in-line solid-phase extraction-liquid chromatography-tandem mass spectrometry. *Journal of Chromatography B* **2017**, *1064*, 124-138.
93. Anastassiades, M.; Lehotay, S. J.; Stajnbaher, D.; Schenck, F. J. Fast and easy multiresidue method employing acetonitrile extraction/partitioning and "dispersive solid-phase extraction" for the determination of pesticide residues in produce. *Journal of AOAC international* **2003**, *86* (2), 412-431.
94. Braganca, I.; Placido, A.; Paiga, P.; Domingues, V. F.; Delerue-Matos, C. QuEChERS: a new sample preparation approach for the determination of ibuprofen and its metabolites in soils. *Science of the Total Environment* **2012**, *433*, 281-289.
95. Lehotay, S. J.; Son, K. A.; Kwon, H.; Koesukwiwat, U.; Fu, W.; Mastovska, K.; Hoh, E.; Leepipatpiboon, N. Comparison of QuEChERS sample preparation methods for the analysis of pesticide residues in fruits and vegetables. *Journal of Chromatography A* **2010**, *1217* (16), 2548-2560.
96. Stubbings, G.; Bigwood, T. The development and validation of a multiclass liquid chromatography tandem mass spectrometry (LC-MS/MS) procedure for the determination of veterinary drug residues in animal tissue using a QuEChERS (QUick, Easy, CHEap, Effective, Rugged and Safe) approach. *Analytica Chimica Acta* **2009**, *637* (1-2), 68-78.
97. Usui, K.; Hashiyada, M.; Hayashizaki, Y.; Igari, Y.; Hosoya, T.; Sakai, J.; Funayama, M. Application of modified QuEChERS method to liver samples for forensic toxicological analysis. *Forensic Toxicology* **2014**, *32* (1), 139-147.

98. Usui, K.; Hayashizaki, Y.; Hashiyada, M.; Funayama, M. Rapid drug extraction from human whole blood using a modified QuEChERS extraction method. *Legal Medicine* **2012**, *14* (6), 286-296.
99. Westland, J. L.; Dorman, F. L. QuEChERS extraction of benzodiazepines in biological matrices. *Journal of Pharmaceutical Analysis* **2013**, *3* (6), 509-517.
100. Matsuta, S.; Nakanishi, K.; Miki, A.; Zaitso, K.; Shima, N.; Kamata, T.; Nishioka, H.; Katagi, M.; Tatsuno, M.; Tsuboi, K.; Tsuchihashi, H.; Suzuki, K. Development of a simple one-pot extraction method for various drugs and metabolites of forensic interest in blood by modifying the QuEChERS method. *Forensic Science International* **2013**, *232* (1), 40-45.
101. Anzillotti, L.; Odoardi, S.; Strano-Rossi, S. Cleaning up blood samples using a modified "QuEChERS" procedure for the determination of drugs of abuse and benzodiazepines by UPLC-MS/MS. *Forensic Science International* **2014**, *243*, 99-106.
102. Dulaurent, S.; El Balkhi, S.; Poncelet, L.; Gaulier, J. M.; Marquet, P.; Saint-Marcoux, F. QuEChERS sample preparation prior to LC-MS/MS determination of opiates, amphetamines, and cocaine metabolites in whole blood. *Analytical and Bioanalytical Chemistry* **2016**, *408* (5), 1467-1474.
103. Dybowski, M. P.; Dawidowicz, A. L. Application of the QuEChERS procedure for analysis of 9-tetrahydrocannabinol and its metabolites in authentic whole blood samples by GC-MS/MS. *Forensic Toxicology* **2018**, *36* (2), 415-423.
104. Pouliopoulos, A.; Tsakelidou, E.; Krokos, A.; Gika, H. G.; Theodoridis, G.; Raikos, N. Quantification of 15 psychotropic drugs in serum and postmortem blood samples after a modified mini-QuEChERS by UHPLC-MS-MS. *Journal of Analytical Toxicology* **2018**, *42* (5), 337-345.
105. Montesano, C.; Vannutelli, G.; Piccirilli, V.; Sergi, M.; Compagnone, D.; Curini, R. Application of a rapid μ -SPE clean-up for multiclass quantitative analysis of sixteen new psychoactive substances in whole blood by LC-MS/MS. *Talanta* **2017**, *167*, 260-267.
106. Johnson, L. A.; Johnson, R. L.; Portier, R. B. Current "legal highs". *Journal of Emergency Medicine* **2013**, *44* (6), 1108-1115.
107. Baumann, M. H.; Volkow, N. D. Abuse of new psychoactive substances: threats and solutions. *Neuropsychopharmacology* **2016**, *41* (3), 663.

108. Kubo, S. i.; Waters, B.; Hara, K.; Fukunaga, T.; Ikematsu, K. A report of novel psychoactive substances in forensic autopsy cases and a review of fatal cases in the literature. *Legal Medicine* **2017**, *26*, 79-85.
109. King, L. A.; Corkery, J. M. An index of fatal toxicity for new psychoactive substances. *Journal of Psychopharmacology* **2018**, 0269881118754709.
110. Ammann, D.; McLaren, J. M.; Gerostamoulos, D.; Beyer, J. Detection and quantification of new designer drugs in human blood: Part 2: Designer cathinones. *Journal of Analytical Toxicology* **2012**, *36* (6), 381-389.
111. OSAC standard practices for method validation in forensic toxicology. *Journal of Analytical Toxicology* **2013**, *37* (7), 452-474.
112. Peters, F. T.; Drummer, O. H.; Musshoff, F. Validation of new methods. *Forensic Science International* **2007**, *165* (2), 216-224.
113. Strickland, E. C.; Cummings, O. T.; Mellinger, A. L.; McIntire, G. L. Development and validation of a novel all-inclusive LC-MS-MS designer drug method. *Journal of Analytical Toxicology* **2018**, *43* (3), 161-169.
114. Graziano, S.; Anzillotti, L.; Mannocchi, G.; Pichini, S.; Busardo, F. P. Screening methods for rapid determination of new psychoactive substances (NPS) in conventional and non-conventional biological matrices. *Journal of Pharmaceutical and Biomedical Analysis* **2018**.
115. Al-Saffar, Y.; Stephanson, N. N.; Beck, O. Multicomponent LC-MS/MS screening method for detection of new psychoactive drugs, legal highs, in urine-experience from the Swedish population. *Journal of Chromatography B* **2013**, *930*, 112-120.
116. Seither, J.; Reidy, L. Confirmation of carfentanil, U-47700 and other synthetic opioids in a Human Performance Case by LC-MS-MS. *Journal of Analytical Toxicology* **2017**, *41* (6), 493-497.
117. Kudo, K.; Usumoto, Y.; Kikura-Hanajiri, R.; Sameshima, N.; Tsuji, A.; Ikeda, N. A fatal case of poisoning related to new cathinone designer drugs, 4-methoxy PV8, PV9, and 4-methoxy PV9, and a dissociative agent, diphenidine. *Legal Medicine* **2015**, *17* (5), 421-426.
118. Lehmann, S.; Schulze, B.; Thomas, A.; Kamphausen, T.; Thevis, M.; Rothschild, M. A.; Mercer-Chalmers-Bender, K. Organ distribution of 4-MEC, MDPV, methoxetamine and α -PVP: comparison of QuEChERS and SPE. *Forensic Toxicology* **2018**, 1-14.

119. Deventer, K. V. E. P.; Van Eenoo, P.; Baele, G.; Pozo, O. J.; Van Thuyne, W.; Delbeke, F. T. Interpretation of urinary concentrations of pseudoephedrine and its metabolite cathine in relation to doping control. *Drug Testing and Analysis* **2009**, *1* (5), 209-213.

APPENDICES

Appendix 1. The compound name, chemical formula, unique in-house identifier, retention time, precursor ion, all product ions and associated collision energies and abundances for all compounds in the final dMRM method separated by mixture number.

Mix #	Compound Name	Chemical Formula	In House ID	RT (min)	Precursor Ion	Product Ion	CE	Abundance
1	25I-NBMD	C18H20INO4	FIU_0379	9.11	442.04	298.1	16	26142
						291	20	96047
						286.1	16	196843
						276	32	32969
						174.1	8	702207
						164.1	28	18476
						145.1	20	376605
						135.1	32	1196209
						105.1	60	73216
						79.1	60	232510
						77.1	60	326616
						51.1	60	24342
	3-Methylbuphedrone	C12H17NO	FIU_0425	7.17	192.13	161.1	8	344234
						161.1	8	141099

					159.1	20	99200
					146.1	16	200785
					144.1	36	376141
					105.1	20	180964
					91.1	48	84682
					77.1	60	126436
					65.1	60	87906
4-APDB	C11H15NO	FIU_0417	6.44	178.11	133.1	20	270092
					120.1	24	24257
					119.1	12	24730
					105.2	28	38710
					103.1	40	27934
					91.1	48	34474
					79.2	40	37856
					77.2	52	85321
					51.2	60	30663
4-fluoro-a-Pyrrolidinobutiophenone	C14H18FNO	FIU_0397	6.74	236.13	165.1	16	267311
					137.1	20	144229
					123	32	157117

					112.1	24	262664
					109.1	28	458875
					95.1	56	207940
					84.1	32	113650
					75.1	60	89763
					70.1	20	99710
					55.1	48	44392
4'-fluoro-a-Pyrrolidinopropiophenone	C13H16FNO	FIU_0399	6.19	222.12	151.1	16	232987
					123.1	24	349431
					103.1	36	209745
					98.1	28	404864
					95.1	52	64672
					84.2	32	40979
					77.1	60	183706
					70.1	16	88531
					56.1	56	138769
					55.7	48	58514
4-hydroxy MET	C13H18N2O	FIU_0749	5.45	219.14191	160	16	118064

					142	32	6053
					132	32	18540
					131.5	36	2504
					117	40	19684
					115	48	75184
					89	60	19173
					77.1	60	7807
					72.1	12	334783
					65.1	60	11123
4-methoxy PV8	C18H27NO2	FIU_0411	8.69	290.20418	219.2	16	485978
					154.2	28	619924
					135.1	32	263495
					121.1	24	896340
					107.1	44	72222
					91.1	60	64198
					84.2	48	175841
					77.2	60	312126
					69.2	60	72277
					55.2	60	65725

4-methoxy-a-Pyrrolidinopentiophenone	C16H23NO2	FIU_0401	7.57	262.17288	191.1	16	530903
					188.2	8	527600
					135	32	232327
					126.1	24	450678
					121.1	24	684502
					107.1	44	56103
					97.1	48	60685
					91.7	60	88344
					84.1	40	125580
					77.1	60	305935
					55.2	56	55004
4-Methyl-a-ethylaminobutiophenone	C13H19NO	FIU_0402	7.36	206.14666	160	16	203080
					159.1	20	305315
					144.1	32	392813
					132.1	20	74175
					130.1	44	62924
					105.1	28	209488
					91.1	48	117533

						77.1	60	100580
						65.1	60	97836
5-fluoro SDB-006	C21H23FN2O	FIU_0440	10.29	339.17944		232.1	20	592602
						206.1	20	404949
						144	40	187950
						132.1	32	99024
						118.1	36	138347
						116.1	60	70792
						91.1	56	826346
						69.2	40	41150
						65.1	60	129623
						55.2	52	6729
5-MAPB	C12H15NO	FIU_0420	6.94	190.11536		159.1	8	861733
						131.1	20	1009047
						129.1	28	27045
						116.1	32	67569
						115.1	52	92822
						103.1	40	48032
						91.1	36	226735

					77.1	56	276351
					65.2	60	92666
					51.2	60	81525
AB-005	C23H32N2O	FIU_0712	9.38	353.25146	256.1	20	66931
					229.1	16	33336
					158	40	11982
					125	20	169410
					112	24	258186
					98	36	291984
					83	32	13003
					70	60	94389
					58.1	56	66682
					55.1	52	51963
AM2233 azepane isomer	C22H23IN2O	FIU_0633	8.45	459.08551	230.9	32	76284
					202.9	60	50645
					112.1	24	1039252
					98.1	32	149448
					84.1	60	22846
					81.1	60	20338

					76.1	60	19551
					70.1	60	68784
					58.1	60	238730
					55.2	60	23624
AM694 N-(5-hydroxypentyl) metabolite	C20H20INO2	FIU_0698	9.98	434.05387	234.1	40	12567
					230.9	20	850211
					220	40	11943
					202.9	56	312614
					186.1	12	69936
					144	56	13714
					130	44	6020
					104.7	60	11314
					104	60	50017
					76	60	156720
a-Pyrrolidinopentiophenone metabolite 1	C15H23NO	FIU_0407	7.81	234.17796	216.2	16	297432
					215.1	8	640731
					173.1	24	106962

					145.1	20	51346
					117.1	28	27466
					103.1	36	23254
					91.1	36	93734
					79.1	40	66749
					77.1	60	73524
					72.1	20	448673
					57.2	28	31397
Benocyclidine	C19H25NS	FIU_0391	8.89	300.17077	226.1	32	7418
					173	32	15404
					147	32	620996
					135	28	17365
					103.1	60	36467
					86.1	4	450750
					81.1	32	77341
					79.1	60	22182
					69.1	60	34782
					67.1	32	27904
Flubromazepam	C15H10BrFN2O	FIU_0678	9.71	332.99605	211	32	745

					206.1	48	628
					184	32	6882
					179.1	56	4337
					105.1	52	3359
					104.1	60	4786
JWH 081 N-(5-hydroxypentyl) metabolite	C25H25NO3	FIU_0516	10.51	388.18344	230.1	28	186558
					185.1	20	2390607
					157.1	48	705961
					144	40	224925
					142	60	297044
					128.3	60	161942
					127	60	456377
					116	60	68151
					114	60	136197
					69.1	40	61715
JWH 200 5-hydroxyindole metabolite	C25H24N2O3	FIU_0531	8.29	401.17869	160.1	36	3014
					155	20	564845

					127	60	252431
					114.1	32	308841
					100.1	60	7421
					86.1	56	7861
					84.1	52	21667
					70.1	60	68606
					68.1	60	3790
					56.2	60	6072
JWH 203	C21H22ClNO	FIU_0534	11.36	340.13899	214.1	28	108406
					188.1	20	172257
					144	44	108807
					132.1	32	44098
					130	52	55877
					125	28	1084924
					118.1	36	23717
					116	60	50535
					99	60	41926
					89.1	60	117666
N-Methyltryptamine	C11H14N2	FIU_0756	5.98	175.1157	144	8	263533

					132	8	54859
					127	32	21000
					117	28	47145
					115	44	37217
					91	44	24577
					90	56	13644
					89	60	21090
					77	48	15813
					65.1	60	13445
NPB-22	C22H21N3O2	FIU_0595	10.95	360.16338	215.1	16	1353981
					145	40	836951
					129.7	60	114
					117	60	103623
					90.1	60	177918
					71.2	36	11694
PB-22 6-hydroxyisoquinoline isomer	C23H22N2O2	FIU_0603	11.16	359.16813	214.1	16	536563
					158	40	6693
					144	44	223678

					130	48	4767
					116	60	68262
					89	60	7707
					71.1	40	3705
					55.1	60	2183
PCMPA	C16H25NO	FIU_0389	8.04	248.19361	159.1	12	683975
					117.1	28	32506
					91.1	40	860073
					81.1	20	127867
					79.1	52	17255
					73.1	24	35540
					67.1	20	20122
					65.1	60	172066
					58.2	28	175286
					55.1	40	11968
THCA-A	C22H30O4	FIU_0453	13.22	359.21441	341.2	12	45770
					234.3	36	103
					219.1	36	9468
					211.3	36	262

2						203.2	36	807
						193.2	24	21943
						69.2	52	1848
						55.2	60	2014
	8-THC	C21H30O2	FIU_0454	12.27	315.22458	135.1	20	16535
						123.1	36	18191
						107.1	36	6320
						93.1	24	10781
						91.1	56	7223
						81.2	48	4993
						77.2	60	7819
						69.2	36	6143
						67.2	56	6716
	2,3-methylenedioxy pyrovalerone	C16H21NO3	FIU_0108	7.29	276.2	175	20	2047695
						149	32	525363
						135	24	2924163
						126.1	32	1693388
						84.1	44	397748
						79.1	48	264551

					77.1	60	669203
					70.1	20	235520
					65.1	60	830244
					55.1	56	235166
25I-NBF	C17H19FINO2	FIU_0378	9.04	416.04445	291	20	681306
					275.9	32	257739
					260.9	48	121283
					164.1	28	149900
					149.1	40	126534
					134.1	40	111284
					121.1	52	78130
					109.1	56	252726
					104.1	52	78022
					91.1	60	135377
2C-T-2	C12H19NO2S	FIU_0146	7.88	242.1	225.1	8	1011348
					210.1	16	132670
					195	24	64849
					164.1	20	85218
					134.1	28	96527

					121	36	48444
					119	36	47882
					91.1	52	162391
					77.1	60	86829
					59	40	51093
2C-T-4	C13H21NO2S	FIU_0020	8.57	256.1	239.1	8	1739095
					197.1	16	1081480
					182	24	429416
					167	36	341836
					164.1	24	214683
					164.1	12	462601
					134.1	36	171171
					121	44	106071
					119	44	89011
					91.1	56	328543
					77.1	60	117332
2-Fluoromethcathinone	C10H12FNO	FIU_0117	11.65	182.1	149	20	323785
					148	36	197216
					123	20	64393

					103	32	83565
					101	56	45896
					77.1	48	111780
					75.1	60	65655
					58.1	36	20636
					51.1	60	44179
3,4-dimethoxy-a-Pyrrolidinopentiophenone	C17H25NO3	FIU_0393	7.09	292.18344	221.1	16	471584
					165	28	200975
					151.1	28	732198
					126.1	24	609709
					107	60	106169
					97.1	52	55242
					84.1	40	113952
					77.1	60	124661
					69.1	60	67566
					55.2	60	60492
3-methyl-a-Pyrrolidinopropiophenone	C14H19NO	FIU_0395	6.88	218.14666	202.2	8	621004

						147.1	16	310958
						146.1	16	407197
						119.1	24	518374
						117.1	36	91511
						98.1	28	332567
						91.1	48	253525
						77.1	60	97633
						70.1	20	100738
						65.1	60	90449
						56.2	56	122555
						55.1	48	55536
	4'-Methyl-N-methylhexanophenone	C14H21NO	FIU_0396	8.35	220.16231	189.1	8	198304
						161.1	12	1426357
						158.1	36	140871
						145.1	24	242869
						144.1	40	268172
						133.1	20	1419465
						131.1	28	85902
						105.1	24	275429

					91.1	48	118296
					77.1	60	111364
4-ethyl-N,N-dimethylcathinone	C13H19NO	FIU_0138	11.65	206.2	151	4	145310
					143.1	16	190223
					123	16	111010
					105.1	28	1181578
					103.1	48	167663
					103.1	28	87754
					91.1	36	138899
					79.1	44	313607
					77.1	60	489298
					72.1	28	1381308
					58.1	32	93347
4-fluorousovathinone (hydrochloride)	C9H10FNO	FIU_0141	11.66	168.1	135.1	24	694
					95	44	4014
					77.1	40	80048
					75.1	60	13026
					51.1	60	28800

4-hydroxy MiPT	C14H20N2O	FIU_0750	5.85	233.15756	160.1	8	3375994
					160	20	141533
					132	32	19884
					131.5	36	2923
					117	44	25703
					115	44	88696
					105	44	6389
					89	60	16625
					86.1	12	315763
					77	60	8381
					65.1	60	9635
4-methylmethcathinone (Mephedrone/4-MMC)	C11H15NO	FIU_0006	6.66	178.1	145.1	20	2302144
					144.1	36	1455458
					130	32	140954
					119	20	309913
					115	52	136339
					103.1	48	158629
					91.1	40	400797
					77.1	60	495361

					65.1	60	262038
A-796260	C22H30N2O2	FIU_0441	9.77	355.23073	125.1	20	1119736
					114.1	32	519024
					100.1	56	15824
					97.1	36	125151
					84.1	52	46333
					83.2	40	44130
					70.1	52	146725
					69.1	44	74507
					57.2	56	137447
					55.2	56	238364
AB-005 azepane isomer	C23H32N2O	FIU_0713	9.5	353.25146	352.1	4	42452
					324.1	12	53475
					253	24	44169
					125	20	45915
					112	24	657114
					98.1	32	20312
					84.1	56	17451
					81	52	11750

					79	60	6790
					70	60	47807
					58.1	56	204217
					56.1	60	7589
					55.1	60	42897
ADB-PINACA isomer 1	C19H28N4O2	FIU_0734	10.73	345.22123	328.2	4	274056
					300.2	12	247817
					215.1	24	314954
					209	24	61
					145	48	177721
					117	60	19264
					90	60	22848
					89.5	60	9735
					71.1	44	3018
AKB48 N-(5-fluoropentyl) analog	C23H30FN3O	FIU_0727	11.85	384.23729	135.1	24	470715
					107	56	41915
					93	60	67050
					91	60	11533
					81.1	60	19852

					79	60	57951
					77	60	11623
					69.1	60	5924
					67.1	60	24116
					55.1	60	9996
EG-018	C28H25NO	FIU_0468	12.73	392.19361	350.1	40	68
					264.1	24	81057
					236.1	36	5998
					233.4	20	69
					194	44	6117
					179.1	52	66432
					166.1	56	13400
					155.1	24	1066831
					127.1	60	762090
					77.2	60	9722
JWH 018 N-(5-hydroxypentyl) metabolite	C24H23NO2	FIU_0484	10.32	358.17288	230.1	28	35272
					208.9	24	108
					155	24	625456

					155	20	1330282
					144	40	61181
					127	52	867032
					116	60	23187
					77.1	60	14406
					69.1	40	17352
JWH 018 N-propanoic acid metabolite	C22H17NO3	FIU_0487	9.97	344.12084	284.2	24	8653
					216.1	24	122192
					144	44	37083
					133.2	16	280
					127.9	60	6280
					127	56	532594
					116.1	60	20701
					77.1	60	16504
					73.1	48	8391
KM 233	C25H30O2	FIU_0459	12.04	363.22458	302.1	16	63
					194.4	60	174
					191.3	32	200
					163.1	32	4167

					135.2	28	3040
					119.1	20	468436
					93.2	44	3440
					91.1	60	289710
					79.2	56	11550
					65.1	60	6694
Loperamide	C29H33ClN2O2	FIU_0764	9.25	477.22306	266.1	24	495262
					238.1	52	18421
					223.1	60	2768
					222.1	60	3490
					210.1	60	234375
					193.1	56	5052
					178	60	7396
					167.1	60	9271
					115	60	18840
					72.1	60	58975
MAM2201 N-pentanoic acid metabolite	C25H23NO3	FIU_0643	10.53	386.16779	244.1	24	52242
					174.1	12	530579
					169.9	24	50012

					169.1	24	697090
					146.1	16	266630
					144	40	52536
					141.8	52	24097
					141.1	48	395051
					130.1	32	326747
					115.1	60	134388
					101.1	36	12951
					83.1	40	15759
					55.1	56	41321
N-Ethylbuphedrone	C12H17NO	FIU_0431	6.46	192.13101	145.1	20	241546
					118.1	24	111048
					117.1	32	64030
					91.1	32	248108
					77.1	60	224389
					65.1	60	72657
					51.1	60	74430
PB-22	C23H22N2O2	FIU_0596	11.18	359.16813	214.1	8	1938796
					158.1	36	22440

					144	40	753166
					130.1	48	15143
					116	60	246065
					89.1	60	31084
					71.1	40	11476
					55.2	60	7274
PCPr	C15H23N	FIU_0390	8.16	218.18305	159.1	8	680438
					117	20	25073
					115	48	14536
					91.1	28	738575
					81.1	16	102919
					79.1	40	12197
					67.1	16	15195
					65.1	60	235075
					60.2	4	488820
					55.1	40	9181
RCS-4 2-methoxy isomer	C21H23NO2	FIU_0085	10.97	322.2	144	36	40072
					135	20	6826092
					120	52	111190

					107.1	40	53326
					105	48	105693
					92	60	751008
					79.1	44	401234
					77.1	60	3790055
					64.1	60	78160
					51.1	60	226015
SER-601	C28H38N2O2	FIU_0463	13.21	435.29333	417.3	16	23680
					284.2	28	181091
					214.1	48	50506
					135.1	32	839440
					107.1	60	99991
					93.1	60	132123
					81.2	60	35563
					79.2	60	115502
					67.2	60	45300
					55.2	60	17024
UR-144 Degradant	C21H29NO	FIU_0645	11.63	312.22491	214.1	20	302746
					206.9	60	69

						158.1	36	4585
						144	40	129095
						130.1	52	3131
						116	60	46762
						89	60	11851
						83.1	32	4550
						71.2	36	2381
						55.2	60	10718
	XLR11 N-(2-fluoropentyl) isomer	C21H28FNO	FIU_0661	11.4	330.21549	312.2	20	11935
						232.1	24	33302
						144.1	44	11056
						130	56	4618
						125.1	24	83427
						97.1	28	17325
						83.1	24	12727
						69.1	44	13842
						57.2	48	19229
					55.1	44	42930	

3	(-)-3,4-Methylenedioxy Pyrovalerone	C16H21NO3	FIU_0356	7.73	276.15214	315.3	4	40769
						247.2	12	39282
						205.1	16	347586
						175.1	20	408998
						175.1	4	58816
						149	32	253614
						135.1	28	383560
						126.1	28	506535
						121	48	140927
						84.1	40	146297
						77.1	60	112730
						65.1	60	297826
						55.1	56	77494
	(+)-WIN 55,212-2 (mesylate)	C27H26N2O3	FIU_0094	10.79	427.2	328.1	28	80845
						299.1	28	62119
						212	36	52652
						200.1	44	72975
						175.1	4	108163

					155	24	5725977
					127	60	2896559
					100.1	48	778004
					72.1	60	62162
					71.1	12	48803
					70.1	60	135203
					56.1	60	126552
(±)-CP 47,497-C8-homolog	C22H36O2	FIU_0098	11.67	333.3	149.1	24	4793
					133	52	8726
					121	28	18823
					107	20	23500
					85.1	12	17066
					71.2	16	17695
					57.1	36	21577
(R)-(-)-JWH 018 N-(4-hydroxypentyl) metabolite	C24H23NO2	FIU_0472	10.33	358.17288	340.2	16	11643
					284.2	24	18984
					230.1	20	11666
					186.2	12	21818

					155.1	20	938068
					144.1	40	32667
					127.1	56	607512
					116.1	60	11799
					77.1	60	10917
					69.2	40	25517
?9-THC	C21H30O2	FIU_0455	12.15	315.22458	193.1	20	46218
					135.2	20	20933
					123.1	36	29699
					107.1	36	11891
					93.1	24	18446
					91.1	60	11394
					81.2	20	11724
					69.2	40	13427
					67.2	56	12520
					55.2	56	10339
2,3-pentylone isomer	C13H17NO3	FIU_0026	7.47	236.1	218.1	8	727720
					188.1	12	1974111
					175.1	12	857458

					159.7	24	604545
					159.1	28	424298
					135	20	667530
					131.1	40	907532
					86.1	20	209571
					77.1	60	454804
					65.1	60	250844
3C-B-fly	C13H16BrNO2	FIU_0315	8.49	298.03644	281	12	193399
					253	24	24972
					202.2	8	598438
					202.1	24	104498
					187.1	36	61286
					173.1	36	39237
					159.1	40	26262
					145.1	52	18648
					131.1	56	22939
					115.1	60	23843
					91.1	60	26066

	4-Methyl-a-ethylaminopentiophenone	C14H21NO	FIU_0403	7.92	220.16231	324.1	12	72894
						175.1	8	216047
						160.1	16	250979
						159.1	20	155713
						144.1	36	398270
						132.1	24	148883
						105.1	24	258233
						91.1	56	156085
						77.1	60	94195
						65.1	60	117031
	AB-FUBINACA	C20H21FN4O2	FIU_0715	10.13	369.16485	352.1	4	57840
						330.1	4	70311
						302.1	12	72370
						253	24	59804
						109	48	60703
	BB-22 8-hydroxyisoquinoline isomer	C25H24N2O2	FIU_0626	11.64	385.18378	240.2	20	341445
						144	44	135629

					116	60	21440
					97.1	40	20753
					69.1	52	11923
					55.2	56	116465
Benzydamine	C19H23N3O	FIU_0772	8.69	310.18411	265.1	12	29173
					174	28	11795
					150.1	24	63
					146	40	4248
					91.1	32	8077
					86.1	16	520989
					85.6	20	52030
					71.1	60	7750
					58.1	56	285414
					56.1	60	4928
Bromazepam	C14H10BrN3O	FIU_0674	8.91	316.00072	288	20	32641
					261	24	24219
					260	36	14712
					209.1	28	70869
					208.1	40	43604

					184	28	19204
					182.1	36	107649
					105.1	52	12246
					80.1	32	18638
					78.1	60	13747
Etaqualone	C17H16N2O	FIU_0760	9.93	265.12626	155	24	4476587
					146	28	171874
					131	40	72970
					130	56	56170
					118	36	33889
					117	48	25580
					106	36	14604
					105	40	23400
					103	56	26953
					79.1	52	47869
					77	60	89059
JWH 019	C25H25NO	FIU_0204	11.87	356.2	228.1	24	820152
					158	36	14899
					144	40	504711

					130	52	14808
					127	56	4018455
					116	60	188942
					89.1	60	26097
					77.1	60	98227
					57.1	44	27368
JWH 073 2'-naphthyl-N-(1,1-dimethylethyl) isomer	C23H21NO	FIU_0049	11.42	328.2	272.2	8	782
					154.9	28	988
					144.2	24	890
					127.1	44	986
JWH 073 6-methoxyindole analog	C24H23NO2	FIU_0504	11.21	358.17288	230.1	24	95875
					174.1	40	63493
					159.1	56	12528
					155	24	2648765
					146.1	52	31299
					131	60	12150
					127	56	1982006
					119.1	60	14586

					77.1	60	44841
					57.1	44	6139
JWH 198	C26H26N2O3	FIU_0529	9.71	415.19434	185.1	24	1071980
					170	52	7293
					157	52	258683
					142	60	85256
					127	60	123126
					114.1	32	430279
					100.1	52	9723
					86.1	52	11111
					84.1	60	33976
					70.1	60	103310
Methylhexanamine	C7H17N	FIU_0249	6.99	116.2	99.1	4	16762
					57.1	12	352833
					55.2	28	1541
MMAI	C11H15NO	FIU_0759	7.27	178.11536	161	8	114350
					146	24	17813
					131	32	14037
					128	32	5207

					115	52	8603
					105.1	20	2191683
					105	24	22136
					103	48	16676
					100	12	2386
					91	36	8894
					77.1	60	21797
N,N-dimethylcathinone	C11H15NO	FIU_0252	5.552	178.1	188.2	24	1405182
					133	12	1388177
					103	36	235494
					79.1	36	410746
					77.1	48	1129270
					72.1	24	1582470
					70.1	48	92944
					58.1	28	128139
					57.7	52	67377
					51.1	60	493223
para-Fluorofentanyl	C22H27FN2O	FIU_0670	7.93	355.21074	234.2	24	137932
					150.1	36	208005

					146.1	32	47935
					134.1	32	142300
					105.1	48	1323016
					103.1	60	149472
					79.2	60	201995
					77.2	60	127959
					57.2	40	43315
PCEEA	C16H25NO	FIU_0388	8.22	248.19361	159.1	8	724788
					117.1	32	29668
					115.1	56	17043
					105.1	28	4746
					91.1	36	837643
					81.1	20	125235
					79.1	56	14687
					67.1	20	18474
					65.1	60	191147
					55.1	40	11641
Phenylpiperazine	C10H14N2	FIU_0259	5.772	163.1	161.1	16	96806
					120	20	1095572

					118	28	227891
					106.1	28	66941
					103	32	110897
					93.1	32	49409
					91.1	40	112231
					77.1	44	585727
					65.1	56	73134
					51.1	60	365911
tetrahydro-Harmine	C13H16N2O	FIU_0701	6.76	217.12626	200	8	95891
					188	8	106388
					185	20	11859
					173	28	38572
					158	36	12319
					156	40	9915
					145	36	17625
					144	48	11702
					132.1	8	278224
					130	48	24165
					103	60	9530

4	(±)-Cannabichromene	C21H30O2	FIU_0435	12.34	315.22458	259.1	12	13764
						233.2	12	10679
						193.1	16	29978
						123	40	10291
						121.1	20	1222329
						109.1	16	4276
						107.1	32	3106
						81.1	16	12887
						69.2	32	11537
						67.1	60	5033
						55.1	60	4706
	(±)-epi CP 47,497	C21H34O2	FIU_0565	14.45	319.25588	167	52	1768
						155.1	28	957268
						137	60	1011
	25T2-NBOMe	C19H25NO3S	FIU_0384	8.51	348.15551	331.1	12	103037
						211.1	16	177422
						196	32	26276
						181	44	15374
						174.1	8	2249439

					134.1	44	16848
					93.1	36	171318
					91.1	52	1091684
					77.1	60	36293
					65.1	60	159936
2-methylethcathinone	C12H17NO	FIU_0119	6.69	192.1	159.1	20	345503
					146	16	902266
					145.1	20	1064793
					144.1	32	1086867
					131.1	28	419458
					130	44	403533
					115	52	165517
					91.1	44	364123
					77.1	60	320344
3,4-methylenedioxy pyrovalerone (MDPV)	C16H21NO3	FIU_0024	7.52	276.2	205.1	16	1080770
					175.1	24	1324664
					149	32	824011
					135	24	1208863
					126.1	28	1595907

					121	48	419197
					84.1	44	464988
					77.1	60	344910
					65.1	60	879407
					55.1	56	246234
3'-4'-methylenedioxy-a-pyrrolidinopropiophenone	C14H17NO3	FIU_0125	6.02	248.1	189.1	4	245662
					177	16	674037
					149	24	574241
					147	24	1585673
					119	36	527575
					98.1	24	2094454
					91.1	48	1099682
					70.1	40	87480
					65.1	60	725244
					56.1	60	537012
					55.6	56	222319
4-acetoxy DiPT	C18H26N2O2	FIU_0745	5.79	303.19943	202	16	132374
					160	28	344673

					142	48	16664
					134	24	4674
					132	48	50606
					117	60	42606
					114.1	16	312530
					105.1	60	13505
					102.1	16	33201
					72.1	32	70273
4-methoxy PCP	C18H27NO	FIU_0387	7.99	274.20926	147.1	36	5712
					121.1	36	213978
					106	60	4664
					91.1	60	28386
					86.1	0	95305
					81.1	24	10763
					79.5	60	3980
					78.1	60	26378
					77.1	60	38397
5-fluoro AMB	C19H26FN3O3	FIU_0708	10.62	364.19582	304.1	12	342091
					233	20	403551

						213	32	132149
						177	36	49611
						171	44	13501
						145	44	219791
						116.9	60	38235
						90	60	50298
						88.9	60	21975
						69.1	40	67911
5-fluoro NPB-22	C22H20FN3O2	FIU_0577	10.42	378.15396		233.1	16	1170458
						213.1	28	341088
						202.1	4	193221
						185.1	36	22004
						177.1	32	140963
						171.1	40	36609
						145	44	592556
						121	44	18387
						117	60	106271
						90.1	60	149594
						69.1	36	167724

5-methoxy-a-Ethyltryptamine	C13H18N2O	FIU_0752	7.12	219.14191	162	8	10255
					160	16	162585
					159.1	4	465869
					148	12	12454
					145	36	57929
					130	44	7229
					117	48	63976
					90	60	27254
					89	60	18770
					58.1	28	7383
6-APB	C11H13NO	FIU_0422	6.9	176.09971	131.1	16	429828
					129.1	24	11710
					116.1	32	34084
					115.1	52	45379
					103.1	40	20205
					91.1	32	106119
					77.2	52	111589
					65.2	60	43537
					51.2	60	46292

A-834735	<chem>C22H29NO2</chem>	FIU_0689	11.02	340.21983	242.1	24	12609
					125.1	24	193742
					99.1	36	12845
					97.1	32	27588
					83.1	36	11888
					81.1	44	5513
					79.1	52	3563
					69.1	40	32027
					57.2	48	33240
					55.2	44	61735
AB-PINACA N-(5-hydroxypentyl) metabolite	<chem>C18H26N4O3</chem>	FIU_0725	9.18	347.20049	231.1	20	30297
					213	28	62387
					175	32	5497
					171	44	6001
					145	48	23199
					131	52	2331
					90	60	3271
					69.1	44	11777

AB-PINACA pentanoic acid metabolite	C18H24N4O4	FIU_0726	9.09	361.17976	344.1	4	55075
					316.1	12	56332
					298.1	20	19586
					245.1	20	19036
					227	36	20998
					217	32	28767
					199	48	4506
					175	44	4950
					145	56	9793
					55.1	60	16211
Acetyl fentanyl	C21H26N2O	FIU_0667	7.32	323.20451	324.3	4	239588
					202.1	20	220466
					188.1	24	1463153
					134.1	28	175597
					132.1	36	261461
					117	60	58630
					105.1	40	1637881
					103.1	60	228792
					79.1	60	338140

					77.1	60	303902
AM2201 N-(3-chloropentyl) isomer	C24H22ClNO	FIU_0636	11.44	376.13899	373.6	20	51
					248.1	24	15892
					212.1	32	5993
					155.1	28	90270
					144.1	44	6273
					127	60	80271
					116	60	1484
					69.1	48	5612
ATM4 4-acetoxy analog	C23H25NO5	FIU_0672	10.02	396.17327	378.2	12	243807
					305.2	16	253412
					249.1	28	225275
					221.1	40	290304
					217.1	40	62553
					206.1	60	69305
					189.1	60	95620
					178.1	60	107687

BB-22 5-hydroxyisoquinoline isomer	C25H24N2O2	FIU_0620	11.64	385.18378	384.1	0	2371
					240.2	24	326734
					188.1	24	1781082
					144	44	160396
					116	60	23928
					97.1	44	22789
					69.2	52	13316
					55.2	60	124549
Butyryl fentanyl	C23H30N2O	FIU_0669	8.29	351.23581	352.3	0	362383
					230.2	24	230241
					146.1	36	86916
					134.1	28	261634
					132.1	36	322205
					105.1	48	1643249
					103.1	60	194889
					79.1	60	252033
					77.1	60	192673
CB-13	C26H24O2	FIU_0176	13.07	369.2	299.1	16	763302

					281.1	28	43985
					252.1	60	31559
					241.1	16	202408
					171	28	1940179
					155	24	2253863
					143	52	659771
					127	60	1738277
					115	60	609693
					77.1	60	27331
CP 47,497-C9-homolog	C23H38O2	FIU_0568	14.51	347.28718	210.8	28	104
					121	24	2295
					107.1	20	2713
					71.1	16	2149
					57.1	32	2818
D2PM	C17H19NO	FIU_0181	7.72	254.2	236.1	12	3588641
					178.1	48	306780
					167.1	32	217205
					165.1	60	322611
					158.1	20	439424

					152	56	266546
					130	32	914499
					117	40	244293
					91.1	40	241134
					77.1	60	275464
Delorazepam	C15H10Cl2N2O	FIU_0675	9.57	305.01702	196.1	60	66
					193.1	48	209
					179.2	60	1705
					165.1	36	2143
					140	32	4973
					99.2	56	235
JWH 200 4-hydroxyindole metabolite	C25H24N2O3	FIU_0530	9.82	401.17869	155	24	673867
					127	60	340709
					114.1	32	734541
					100.1	56	13405
					86.1	52	17245
					84.1	52	52158
					70.1	60	143643
					68.1	60	9477

					58.1	60	7900
					56.1	60	10934
JWH 203 N-(5-hydroxypentyl) metabolite	C21H22ClNO2	FIU_0536	10.22	356.13391	230.1	28	9102
					204.1	16	109231
					186.1	16	184811
					144.1	48	26584
					130	36	46178
					125	28	542463
					118	28	10319
					99	60	16453
					89.1	60	43399
					69.1	36	14538
JWH 387	C24H22BrNO	FIU_0550	12.06	420.08848	233	28	511810
					214.1	28	84677
					205	52	305239
					144	52	53243
					126	60	224465
					116.1	60	16024

	Methiopropamine	C8H13NS	FIU_0308	5.52	156.07687	125	8	283684
						97	24	374348
						91.1	24	18916
						81.1	28	20771
						79.1	36	17333
						69.1	48	11566
						66.1	48	11342
						65.1	56	15550
						58.2	8	359989
						53.1	48	98382
	PB-22 N-(4-hydroxypentyl)-3-carboxyindole metabolite	C14H17NO3	FIU_0609	8.83	248.12084	230.1	4	11815
						186	8	5420
						174	20	3847
						157.1	28	188
						130	32	3473
						128.1	44	641
						93	12	92
						77	60	1812

					69.1	20	2403
UR-144	C21H29NO	FIU_0268	11.85	312.2	214.1	24	928611
					144	36	589452
					130	56	129459
					125.1	20	2475727
					116	60	241218
					97.1	28	552399
					83.1	24	326052
					69.1	40	375001
					57.1	48	639495
					55.1	40	1224554
XLR12	C20H24F3NO	FIU_0666	11.23	352.181	353.3	0	351590
					254.1	28	326414
					144	48	177183
					125.1	24	806231
					116	60	138509
					97.1	32	164250
					83.1	24	153361
					69.1	44	113809

5	(±)-ORG 28611	C23H33N3O2	FIU_0436	9.37	384.25728	57.2	52	200408
						55.2	48	390987
						338.3	8	4540
						270.1	16	1394881
						174.1	40	730840
						159	60	311991
						146.1	52	12847
						131	60	27362
						118.1	60	10297
						97.1	40	65038
						69.2	52	38785
						55.1	60	364862
	25I-NBOMe 4-methoxy isomer	C18H22INO3	FIU_0381	8.91	428.06444	301.2	8	25474
						272.2	16	16838
						121.1	20	1768680
						106.1	60	22922
						93.1	60	9792
						91.1	60	121035
						78	60	76861

					77.1	60	183073
					65.1	60	12998
					55.1	60	6140
2C-E	C12H19NO2	FIU_0014	8.43	210.1	193.1	8	1855745
					178.1	16	738861
					163.1	28	369121
					135.1	20	175389
					115	52	89190
					105.1	28	289192
					103.1	40	103167
					91.1	52	274033
					79.1	40	174784
					77.1	60	309902
2C-I	C10H14INO2	FIU_0025	8.18	308	291	8	1001652
					276	20	327561
					260.9	32	149200
					164.1	20	159682
					149	28	141339
					134.1	32	154742

					106	52	219173
					105.6	60	61119
					91.1	56	321837
					78.1	60	215637
2-methoxymethcathinone	C11H15NO2	FIU_0118	6.24	194.1	176.1	8	991788
					161	20	569396
					146	28	152952
					144	36	104320
					132	36	122990
					118	40	191448
					117	56	107569
					91.1	52	109691
					77.1	60	160028
					58.1	20	75381
3-fluoromethcathinone (hydrochloride)	C10H12FNO	FIU_0131	11.65	182.1	164.1	12	1605492
					149	20	1119625
					148	36	758213
					123	20	187934
					103.1	32	259411

					101	56	168349
					95	52	76280
					77.1	48	352149
					75	60	239356
					51.1	60	129921
4-fluoro PV8	C17H24FNO	FIU_0409	8.67	278.18419	207.2	20	95752
					154.2	28	382888
					137.1	20	98781
					123.1	32	232441
					109.1	24	651031
					95.1	60	264425
					84.2	44	164865
					72.2	32	56197
					70.2	20	109277
					55.2	56	46963
4-fluoro PV9	C18H26FNO	FIU_0410	9.16	292.19984	177.1	16	570049
					168.2	32	406329
					137.1	20	90019
					125.1	20	100235

					123.1	36	225314
					109.1	28	841614
					95.1	60	268112
					84.2	48	171654
					72.2	36	75278
					70.2	20	114176
					55.2	56	79203
4-methoxy-a-Pyrrolidinobutiophenone	C15H21NO2	FIU_0400	6.97	248.15723	149.1	20	389778
					135	28	200754
					121.1	32	419348
					112.1	24	435277
					91.1	52	106767
					84.1	36	85157
					77.1	56	264095
					70.1	48	77796
					55.2	52	64271
4-methyl-a-pyrrolidinobutiophenone (HCl)	C15H21NO	FIU_0148	7.4	232.2	161.1	16	1488009

					133.1	16	531978
					119	28	681065
					112.1	28	976569
					105.1	28	2205142
					91.1	48	964711
					84.1	32	377228
					77.1	60	314627
					70.1	44	218660
					65.1	60	506222
5-fluoro ADBICA	C20H28FN3O2	FIU_0706	10.26	362.21656	345.2	8	262237
					273.6	4	53
					232.1	20	297629
					144	48	129991
					116	60	45854
					89	60	4351
					69.1	48	10037
5-fluoro PB-22 8-hydroxyisoquinoline isomer	C23H21FN2O2	FIU_0589	10.71	377.15871	232.1	20	2515332
					212.1	40	24396

					176.1	40	15269
					161.1	8	255598
					158	40	19570
					144	48	1168634
					130	60	19029
					116	60	430178
					89.1	60	35737
					69.1	44	93366
					61.1	60	15522
6-APDB	C11H15NO	FIU_0423	6.45	178.11536	133.1	20	216061
					120.1	28	11970
					105.1	28	29797
					103.1	40	18627
					100.2	4	6466
					91.1	48	19956
					79.2	40	27938
					77.1	52	65486
					51.2	60	20410
Acetyl norfentanyl	C13H18N2O	FIU_0668	5.8	219.14191	136.1	16	54754

					94.1	36	38367
					84.1	16	925546
					82.1	32	11214
					77.1	60	24360
					67.1	28	24080
					57.1	28	7642
					56.2	28	228000
					55.1	44	338411
					53.1	60	14730
ADB-PINACA	C19H28N4O2	FIU_0733	10.92	345.22123	344.2	4	93835
					328.2	4	270236
					316.1	12	98571
					300.1	12	277681
					232.1	20	5162
					215	24	343729
					145	48	203544
					125.8	36	50
					117	60	21225
					90	60	24901

					89.5	60	12483
					71.1	44	2929
ADB-PINACA N-(5-hydroxypentyl) metabolite	C19H28N4O3	FIU_0738	9.63	361.21614	231	20	44215
					213	32	82008
					188.1	8	465922
					185.1	40	3689
					175	36	8114
					171	48	8589
					145	52	32910
					131	52	3603
					69.1	48	17081
a-Ethylaminopentiophenone	C13H19NO	FIU_0404	7.17	206.14666	146.1	16	251766
					130.1	36	254640
					118.1	24	164429
					117.6	36	65938
					105.1	24	57604
					91.1	32	255679

					77.1	60	236677
					65.1	60	79354
					51.1	60	75160
AKB48 N-(4-fluorobenzyl) analog	C25H26FN3O	FIU_0740	11.94	404.20599	135.1	20	669326
					107	60	55132
					93	60	88456
					91	60	12840
					81.1	60	25167
					79.1	60	74219
					77	60	13774
					69.1	60	8223
					67.1	60	27495
					55.1	60	12570
BB-22 6-hydroxyisoquinoline isomer	C25H24N2O2	FIU_0622	11.52	385.18378	240.2	20	501239
					144	44	193649
					138.5	24	64
					116	60	30511

					97.1	40	31800
					69.1	48	18234
					55.2	60	173871
JWH 193	C26H26N2O2	FIU_0528	9.86	399.19943	169.1	20	1001650
					141	56	424479
					114.1	32	459911
					100.1	60	10409
					86.1	52	11791
					84.1	56	38421
					70.1	60	111000
					68.1	60	6438
					58.1	60	7454
					56.2	60	8185
JWH 210	C26H27NO	FIU_0225	12.095	370.2	214.1	24	1230945
					183.1	28	4075202
					155.1	44	1247308
					153.1	56	1045567
					144	44	659238
					129	60	388347

					128.2	60	219700
					116	60	222546
					115	60	419288
					77.1	60	197334
JWH 210 5-hydroxyindole metabolite	C26H27NO2	FIU_0539	11.32	386.20418	230.1	24	92449
					183.1	28	374825
					178	20	146366
					160	44	59085
					155	44	121471
					153	60	93409
					132	60	21012
					129.1	60	32997
					127.9	60	14656
					115	60	34942
					77.1	60	17021
Levamisole	C11H12N2S	FIU_0763	5.55	205.07212	149	4	2033
					130	44	13591
					128	36	20736
					123	28	44966

					117	28	28490
					103	40	16127
					91	40	99914
					77	56	44320
					65	60	34975
PB-22 3-carboxyindole metabolite	C14H17NO2	FIU_0597	10.51	232.12593	188.1	8	8886
					186.3	20	116
					144	28	1355
					132	16	10470
					118	20	11328
					91	44	4748
PB-22 N-(4-hydroxypentyl) metabolite	C23H22N2O3	FIU_0608	10.06	375.16304	281.6	60	115
					230.1	12	1439072
					225.3	8	107
					214.1	52	87
					144	36	686688
					116	60	135422
					89.1	60	13211

					87.1	32	19830
					69.1	40	410011
					67.1	56	7095
Pentedrone metabolite ((±)- Ephedrine stereochemistry)	C12H19NO	FIU_0341	7.6	194.14666	176.1	8	723346
					145.1	16	45243
					133.1	24	126644
					132.1	32	55838
					117.1	24	38017
					91.1	40	112699
					79.1	36	21077
					77.1	60	49015
					65.1	60	34924
					56.1	40	31100
RCS-8 4-methoxy isomer	C25H29NO2	FIU_0088	11.73	376.2	254.1	32	496131
					149	24	1420456
					144	48	435283
					135	36	1808051
					121	36	4720971

						107	56	309871
						91.1	60	401812
						77.1	60	1203244
						69.1	52	364009
						55.1	60	261828
6	(+)-3,4-Methylenedioxy Pyrovalerone	C16H21NO3	FIU_0357	7.73	276.15214	205.1	16	342544
						175.1	20	397365
						149	32	238258
						135	28	367160
						126.1	28	475979
						121	48	127849
						84.1	44	144399
						77.1	60	107016
						65.1	60	290472
						55.1	56	73340
	(+/-)-WIN 55,212 (mesylate)	C27H26N2O3	FIU_0101	10.77	427.2	340.1	28	20420
						328.1	28	25770
						299.1	28	19194

					212.1	36	16268
					200.1	44	24450
					155	24	1749417
					127	60	810686
					100.1	48	243642
					70.1	60	44863
					56.1	60	43262
(±)-JWH 073 N-(3-hydroxybutyl) metabolite	C23H21NO2	FIU_0510	10.3	344.15723	284.1	24	22739
					216.1	24	36391
					158.1	32	25448
					155	20	759571
					155	16	1825048
					144	44	17895
					130	48	23135
					127	56	581475
					116	60	9723
					77.1	60	16256
					55.2	56	8530
1'-naphthoyl indole	C19H13NO	FIU_0107	10.09	272.1	254.1	20	13489

					179.1	8	834875
					144	20	1064251
					127	40	1571228
					116	48	375694
					101	60	60558
					89.1	60	343881
					77.1	60	329137
					63.1	60	30423
					51.1	60	16941
2,5-DMMA	C12H19NO2	FIU_0304	7.25	210.14158	164.1	20	328314
					151.1	16	621865
					149.1	32	187888
					123.1	24	58603
					121.1	28	310579
					91.1	40	225177
					78.1	60	139118
					77.1	56	192965
					65.1	60	117420
2C-T-7	C13H21NO2S	FIU_0312	9.07	256.1293	239.1	8	513065

					224.1	20	42920
					197.1	20	67597
					182	28	58606
					167	36	76734
					164.1	24	48230
					134.1	32	45939
					121.1	44	22386
					119	48	20742
					91.1	56	73590
2C-TFM	C11H14F3NO2	FIU_0313	8.53	250.09766	233.1	8	161749
					218	20	105955
					203.1	8	641016
					203	36	56012
					175.1	12	586087
					151	40	6082
					133.1	36	12825
					127	56	23931
					121.1	24	550540
					115.1	40	7765

					113	48	20627
					91.1	60	14144
					77.1	60	13527
2-methoxy Ketamine	C14H19NO2	FIU_0283	7.03	234.14158	135.1	24	38646
					115.1	60	35198
					93.1	32	51285
					91.1	48	459790
					77.1	60	89389
					67.2	28	108334
					65.2	60	167281
3,4-EDMC	C12H15NO3	FIU_0285	6.28	222.10519	204.1	12	773196
					189.1	20	314874
					163.1	24	72046
					148.1	28	197052
					133	36	227621
					105.1	52	73840
					91.1	44	116514
					77.1	60	96040
					65.1	60	113162

					58.2	12	103344
3-Bromoamphetamine	C9H12BrN	FIU_0286	8.02	214.01531	197	4	56464
					168.9	16	102523
					120.9	16	100
					118.1	20	11776
					117.1	40	23442
					115.1	60	8202
					91.1	60	14528
					90.1	40	40223
					89.1	60	35928
					64.1	60	3923
3-Methoxyamphetamine	C10H15NO	FIU_0289	6.83	166.11536	149.1	4	398894
					121.1	16	443724
					106.1	36	9707
					91.1	32	143466
					78.1	48	90145
					77.1	36	63633
					65.1	48	78124
					63.1	60	9719

					52.1	60	27862
					51.1	60	24061
4-quinolone-3-carboxamide CB2 ligand	C26H34N2O3	FIU_0151	12.72	423.3	272.6	24	846128
					202	44	368765
					187	60	145440
					135.1	28	5354440
					107.1	60	588428
					93.1	60	843037
					81.1	60	231173
					79.1	60	728317
					77.1	60	123049
					67.1	60	271053
5-fluoro JWH 018 adamantyl analog	C24H30FNO	FIU_0474	11.62	368.23114	135.2	36	1063440
					107.1	56	140480
					93.1	60	218304
					91.1	60	38066
					81.2	60	59976
					79.2	60	207437

					77.1	60	40949
					69.2	60	18282
					67.2	60	72180
					55.2	60	29403
5-fluoro PB-22 7-hydroxyisoquinoline isomer	C23H21FN2O2	FIU_0587	10.55	377.15871	232.1	24	1679788
					212.1	44	20280
					176.1	44	12071
					158	40	18539
					144	48	1042519
					130.1	56	16766
					116	60	346830
					89.1	60	31060
					69.2	48	71486
					61.1	60	12336
AM1248 azepane isomer	C26H34N2O	FIU_0456	9.86	391.26711	135.2	32	241646
					112.2	28	496340
					107.1	56	24952
					98.1	44	37564

						93.2	60	43626
						81.1	60	17735
						79.1	60	34146
						70.2	60	32347
						58.2	60	141103
						55.2	60	18752
	a-Pyrrolidinobuthiothiophenone	C12H17NOS	FIU_0406	5.82	224.10308	153	12	190553
						125	20	179496
						112.1	20	611336
						111	36	111764
						97	36	144145
						84.1	36	56497
						83.1	60	32636
						70.1	44	91687
						56.4	60	25731
						55.2	52	74472
	CB-86	C26H43NO3	FIU_0179	11.84	418.3	361.3	16	295441
						292.2	12	268271

					275.1	16	293382
					182.1	16	213452
					142.1	16	240826
					97.1	24	87608
					85.1	32	196331
					71.1	32	387338
					58.1	28	2599650
					55.1	60	314099
DOI	C11H16INO2	FIU_0299	8.73	322.02257	305	8	419743
					277	20	113922
					178.1	20	163710
					163.1	28	89350
					135.1	36	110861
					105.1	52	99762
					103.1	60	44843
					91.1	60	62462
					79.1	60	48173
					77.1	60	101358
JWH 213	C27H29NO	FIU_0543	12.06	384.22491	228.1	28	362663

					183.1	28	1846841
					158	44	231505
					155.1	44	559364
					153.1	56	467202
					130	60	68152
					129.1	60	181442
					127.8	60	74762
					115.1	60	175718
					77.1	60	73776
JWH 251 4-methylphenyl isomer	C22H25NO	FIU_0077	11.42	320.2	214.1	24	754154
					188.2	20	199026
					144.1	40	567236
					130.1	48	134294
					119	24	419304
					116.1	60	249506
					105.1	24	1828336
					91.1	56	267202
					79.1	60	266183
					77.1	60	245281

Lisdexamfetamine	C15H25N3O	FIU_0301	5.63	264.19976	280.2	0	232757
					247.2	12	24768
					136.1	12	9050
					129.1	8	19886
					119.1	20	8899
					91.1	40	22812
					85.2	28	136
					84.1	24	110392
					67.1	44	4084
					56.1	60	29628
Mepirapim	C19H27N3O	FIU_0329	9.29	314.21541	214.1	12	1082746
					158.1	32	12956
					144.1	36	425625
					130.1	44	8942
					116.1	60	165617
					91.1	32	193
					89.1	60	39360
					71.2	36	7366
					55.2	52	4746

Mescaline	C11H17NO3	FIU_0319	6.1	212.12084	195.1	4	221645
					180.1	16	51089
					165.1	20	38973
					135	28	9649
					133.1	28	17840
					105.1	40	10825
					91.1	48	22622
					79.1	40	11814
					77.1	56	43236
					65.1	60	15648
MT-45	C24H32N2	FIU_0328	9.61	349.25655	181.1	24	1160727
					179.1	40	88903
					169.1	20	393925
					167.1	20	91623
					166.1	40	398058
					165.1	60	300109
					153.1	40	46172
					149.1	8	938291
					103.1	56	371135

					87.1	32	115883
					77.1	60	129098
para-Methoxymethamphetamine	C11H17NO	FIU_0307	6.64	180.13101	121	20	680782
					119	36	14331
					93.1	24	20217
					91.1	36	204015
					78.1	52	161368
					77.1	40	119965
					65.1	56	114966
					52.1	60	29479
					51.1	60	38474
PB-22 7-hydroxyisoquinoline isomer	C23H22N2O2	FIU_0605	11.22	359.16813	214.1	24	1693224
					158	36	27828
					144	44	975805
					143.2	56	2784
					130.1	56	20429
					116	60	290169

					89.1	60	30487
					71.2	40	15875
					55.2	60	8068
Pentedrone metabolite ((±)-Pseudoephedrine stereochemistry)	C12H19NO	FIU_0342	7.51	194.14666	176.1	8	802809
					145.1	16	49571
					133.1	24	151368
					132.1	32	68685
					117.1	20	43358
					104.1	36	29306
					91.1	44	126771
					77.1	60	35084
					65.1	60	39991
					56.1	40	41050
Propylhexedrine	C10H21N	FIU_0302	8.31	156.1674	125.1	8	21793
					83.1	16	116302
					69.2	16	371871
					67.2	12	5018
					57.2	12	52422

					55.2	28	193989
					53.2	52	6437
UR-144 N-(2-hydroxypentyl) metabolite	C21H29NO2	FIU_0648	11.01	328.21983	230.1	24	30608
					144	40	23786
					130.1	48	6696
					125.1	24	152328
					116	60	10349
					97.1	32	25740
					83.1	24	16343
					69.1	40	22507
					57.2	48	33477
					55.1	44	67066
UR-144 N-(5-methylhexyl) analog	C23H33NO	FIU_0656	12.29	340.25621	322.3	20	17752
					242.2	24	41749
					144	40	23285
					130.1	60	6577
					125.1	24	140806

7						97.1	32	29712
						83.1	28	16883
						69.1	40	19898
						57.2	48	43015
						55.2	48	67582
	(R)-(-)-MT-45	C24H32N2	FIU_0324	9.6	349.25655	181.1	24	1251652
						179.1	44	98594
						169.2	16	464387
						167.2	20	103109
						166.1	40	464629
						165.1	60	355992
						153.1	40	54038
						103.1	56	428647
						87.1	36	135549
						77.1	60	140327
	2,3-Dichlorophenylpiperazine	C10H12Cl2N2	FIU_0326	8.49	231.03775	188	20	131580
						152.7	28	73679
						152	36	66808
						118.1	40	28664

					117.1	56	58659
					90.7	56	21105
					90.1	60	12302
					89.6	60	12413
					75.1	60	13446
					70.1	20	13990
2C-T	C11H17NO2S	FIU_0311	7.66	228.098	211.1	8	561379
					196	20	101653
					181	24	64240
					166	20	58717
					164.1	20	47394
					134.1	24	73037
					121	36	31062
					119	36	33248
					91.1	48	88998
					77.1	60	51757
3,4-Dimethylethcathinone	C13H19NO	FIU_0331	7.98	206.14666	188.2	12	762836
					173.1	20	295598
					159.8	16	169002

					159.1	20	326413
					158.1	32	395250
					144.7	24	106532
					144.1	36	105431
					115.1	60	106431
					105.1	36	93991
					91.1	56	106950
3-Bromomethcathinone	C10H12BrNO	FIU_0352	7.41	242.01023	145.1	16	364113
					144.1	36	238510
					132.1	20	22824
					131	40	15869
					128	56	18496
					104	36	11727
					103.1	60	27212
					78	60	17422
					77.1	60	43051
					58.2	60	7153
3C-P	C14H23NO3	FIU_0316	8.29	254.16779	237.1	4	175322
					195.1	12	217228

					167.1	20	46877
					163.1	16	47645
					149.1	4	625482
					135.1	20	25931
					107.1	28	83462
					103.1	40	15716
					91.1	60	26379
					79.1	40	24030
					77.1	60	53173
4-Methoxyamphetamine	C10H15NO	FIU_0293	6.69	166.11536	121.1	16	403590
					119	36	10174
					93.1	20	6156
					91.1	32	118106
					78.1	48	96133
					77.1	40	72439
					65.1	52	75461
					52.1	60	24971
					51.1	60	26844
4-methoxy-N,N-Dimethylcathinone	C12H17NO2	FIU_0332	6.47	208.12593	163.1	12	268429

					135.1	20	374350
					105.1	32	148883
					103.1	40	75961
					91.1	60	93866
					79.1	40	105894
					77.1	56	177530
					72.2	20	649795
					70.1	52	34223
					58.2	28	22286
5-fluoro NNEI	C24H23FN2O	FIU_0437	10.55	375.17944	232.2	20	801759
					212.2	40	8266
					206.2	16	18743
					176.1	40	4557
					158.1	44	7466
					144.1	48	406051
					130.1	60	7367
					116.1	60	137737
					89.1	60	11688
					69.2	44	36116

9-octadecenamide (oleamide)	C18H35NO	FIU_0157	11.72	282.3	111.1	12	35047
					97.1	16	57377
					95.1	16	28919
					83.1	20	62044
					81.1	20	25419
					71.1	20	31999
					69.1	24	84689
					67.1	36	16158
					57.1	28	75143
					55.1	40	106311
BB-22 4-hydroxyquinoline isomer	C25H24N2O2	FIU_0619	11.87	385.18378	240.1	12	679498
					158	40	2631
					144	40	242106
					125.4	52	63
					116	60	40566
					97.1	40	33999
					89.1	60	3288
					69.1	52	19312

					55.1	60	198022
Cathine	C9H13NO	FIU_0323	5.68	152.09971	134.1	4	399942
					117.1	16	129016
					115.1	24	101578
					91.1	36	86537
					89.1	52	7744
					77.1	40	7708
					65.1	52	39152
					63.1	60	7549
					56.1	16	23121
					51.1	60	10832
Diclofensine	C17H17Cl2NO	FIU_0298	9.85	322.06872	291	20	38008
					279	20	85701
					256.1	28	27313
					252.1	8	1527188
					209.1	36	21371
					178.1	60	39973
					165.1	60	24296
					159	32	23787

					121.1	24	178112
					91.1	52	48760
					77.1	52	19798
FUB-PB-22	C25H17FN2O2	FIU_0594	10.76	397.12741	301.1	20	106
					224.1	28	11390
					109	44	1409363
					83.1	60	34895
HMA	C10H15NO2	FIU_0300	5.21	182.11028	165.1	4	222342
					137	16	60366
					133.1	16	48909
					105.1	24	56550
					103.1	32	15992
					94.1	44	14357
					79.1	32	30876
					77.1	48	47161
					65.1	52	11638
					51.1	60	20746
JWH 018 2-hydroxyindole metabolite	C24H23NO2	FIU_0475	12.51	358.17288	340.2	16	56591
					270.1	24	99942

					254.2	56	347
					252.6	40	838
					252.1	40	49856
					251.1	60	24784
					230.1	20	9346
					155.1	20	15972
					127.1	56	11992
JWH 251 3-methylphenyl isomer	C22H25NO	FIU_0076	11.41	320.2	214.1	24	2540962
					188.2	16	607795
					144	40	1658027
					130.1	48	159603
					119	24	269801
					116	60	710628
					105.1	24	2951181
					91.1	56	210814
					79.1	60	355442
					77.1	60	348348
MBZP	C12H18N2	FIU_0327	5.45	191.147	160.1	8	765293
					100.1	16	39905

					99.1	12	247295
					91.1	24	1169459
					84.1	24	9708
					70.1	28	62134
					65.1	56	397011
					63.1	60	30028
					58.2	40	115136
					56.1	28	42801
					51.1	60	18793
Mephedrone	C11H15NO	FIU_0337	6.9	178.11536	224.1	12	435090
					145.1	20	580164
					144.1	36	376708
					130.1	32	39373
					119.1	24	86636
					115.1	52	36706
					103.1	48	42353
					91.1	36	104420
					77.1	60	133572
					65.1	60	65730

NRG-3	C16H19NO	FIU_0340	9.12	242.14666	211.1	12	159337
					194.1	36	80735
					181.7	16	325438
					181.1	24	343752
					180.1	40	202246
					167.1	32	114928
					141.1	24	209455
					127.1	56	84986
					115.1	60	111817
RCS-4 N-(4-hydroxypentyl) metabolite	C21H23NO3	FIU_0574	14.51	338.16779	111.1	20	19492
					97.1	24	31254
					95.1	20	16121
					83.1	24	30687
					81.1	24	13559
					71.1	28	19382
					69.1	36	41678
					67.1	40	9018
					57.2	40	37105
					55.1	52	51393

8	UR-144 N-(2-chloropentyl) analog	C21H28ClNO	FIU_0647	11.64	346.18594	248.1	24	24585
						144	40	15950
						130.1	56	3887
						125.1	24	77720
						116.1	60	6232
						97.1	32	15258
						83.1	24	10781
						69.1	44	16166
						57.2	56	17681
						55.1	48	37963
	(-)-(S)-Cathinone	C9H11NO	FIU_0344	5.59	150.08406	133.2	8	70853
						117.1	24	166242
						105.1	16	88675
						103.1	32	14783
						89.8	40	54402
						89.1	52	51167
						79.1	32	23572
						77.1	40	74263
						51.1	60	55674

	(±)-Ethylphenidate	C15H21NO2	FIU_0279	8.12	248.16	174.1	24	12580
						163.1	8	450428
						129.1	40	4034
						115.1	60	7099
						91.1	60	23219
						84.1	20	1404865
						70.2	48	4475
						69.2	60	10177
						67.2	60	22288
						65.2	60	7635
						56.1	60	334424
	2,3-MDA	C10H13NO2	FIU_0358	7.05	180.09463	174.1	8	920493
						135	16	297130
						133.1	16	74445
						105.1	24	177076
						103.1	32	38437
						91.1	40	7465
						79.1	32	90323
						77.1	44	191386

					65.1	44	12707
					51.1	60	116146
2,4-Dimethylmethcathinone	C12H17NO	FIU_0349	7.86	192.13101	159.1	20	669562
					158.1	32	359343
					144.1	32	177906
					116.8	52	21510
					115.1	56	86963
					105.1	32	35737
					91.1	60	81412
					77.1	60	65270
					58.2	12	67072
30C-NBOMe	C20H26ClNO5	FIU_0385	8.35	396.14995	181.1	12	1313212
					151	56	22893
					148	48	235340
					137.1	60	78969
					123.1	60	26954
					120.1	56	23410
					107.1	60	24969
					91.1	60	50605

					90.5	60	31829
					77.1	60	25079
4-Fluoromethcathinone metabolite ((±)- Pseudoephedrine stereochemistry)	C10H14FNO	FIU_0355	6.14	184.10594	166.1	8	957374
					151.1	20	163911
					135.1	20	123112
					133	36	56834
					122.1	36	11460
					115.1	28	107652
					109	36	141519
					83.1	60	67403
					70.1	20	35481
					56.1	40	19331
4-Methylethcathinone metabolite ((±)-Ephedrine stereochemistry)	C12H19NO	FIU_0333	7.32	194.14666	176.1	8	982397
					161.1	20	81004
					147.1	20	82802
					146.1	28	75159

					131.1	20	131292
					116.1	32	52983
					115.1	52	69475
					105.1	24	44898
					91.1	36	151734
5-fluoro PB-22 N-(3-fluoropentyl) isomer	C23H21FN2O2	FIU_0591	10.85	377.15871	232.1	12	335201
					212.1	36	23466
					176	44	4804
					158	40	6443
					148	60	2965
					144	48	85426
					130	56	5878
					116	60	33197
					69.1	52	32572
					61	60	3730
A-836339	C16H26N2O2S	FIU_0442	10.24	311.1715	352	4	99027
					253	20	72417
					187.1	16	2131532
					155.1	36	144832

					129.1	40	150865
					125.1	24	432296
					97.2	36	112367
					87.1	56	50246
					69.2	44	83446
					59.2	44	358874
					57.2	56	210341
					55.2	48	310195
AB-FUBINACA isomer 5	C20H21FN4O2	FIU_0720	9.8	369.16485	324.1	12	32111
					109	44	67569
AM1220	C26H26N2O	FIU_0159	8.79	383.2	286.1	20	662973
					177.1	12	33086
					158	36	137799
					155	32	1020648
					127	60	730738
					112.1	20	2311684
					98.1	40	2882892
					84.1	60	43078
					70.1	60	675347

					58.1	60	451422
					55.1	60	67784
bk-2C-B	C10H12BrNO3	FIU_0314	7.58	274.00006	257	4	15844
					178	12	30634
					163.1	32	14816
					162.1	28	30711
					134.1	40	7210
					119	60	10714
					105	44	4845
					91.1	60	6924
					77.1	60	11872
JWH 018 2'-naphthyl-N-(1-methylbutyl) isomer	C24H23NO	FIU_0037	11.71	342.2	272.1	20	61542
					214.1	20	320866
					155.1	24	3961741
					144	40	908068
					127.1	56	2875178
					116	60	408235
					101	60	14966
					89.1	60	90970

					77.1	60	73585
					71.2	32	14648
JWH 250 N-(5-hydroxypentyl) metabolite	C22H25NO3	FIU_0546	10.02	352.18344	204.1	16	30070
					186.1	12	59991
					144.1	40	22438
					131.1	40	13908
					130.1	44	38757
					121.1	20	509301
					93.1	40	46476
					91.1	56	296378
					69.2	40	16174
					65.1	60	25483
JWH 309	C30H27NO	FIU_0231	12.445	418.2	290.2	20	35097
					220.1	40	16795
					192.1	52	7816
					189	24	4001654
					165.1	60	14172
					155	20	4777497

					127	60	3441735
					101	60	7583
					77.1	60	38110
JWH 398 6-chloronaphthyl isomer	C24H22ClNO	FIU_0081	11.91	376.2	214.1	24	603340
					161	52	3533857
					158	36	9891
					149.1	20	10567
					144	40	347931
					130.1	48	9133
					126	60	918657
					116	60	112090
					89.1	60	15544
NNEI	C24H24N2O	FIU_0447	11.05	357.18886	214.2	20	733015
					188.2	16	16676
					158.1	44	10094
					144.1	44	323174
					132.1	28	3926
					130.1	52	7543
					116.1	60	99833

					89.1	60	10298
					71.2	40	6795
					55.2	60	4279
Pravadoline	C23H26N2O3	FIU_0260	9.295	379.2	135	20	4442637
					114.1	36	710049
					107	52	437431
					100.1	56	24517
					92	60	240906
					86.1	48	25525
					84.1	52	76314
					79.1	60	78822
					77.1	60	964849
					70.1	60	249950
PV9	C18H27NO	FIU_0414	8.99	274.20926	168.2	32	227170
					119.1	20	101200
					107.1	20	87006
					105.1	32	191455
					91.1	28	666212
					84.2	40	125960

9	(R)-(-)-JWH 073 N-(3-hydroxybutyl) metabolite	C23H21NO2	FIU_0511	10.3	344.15723	77.1	60	314657
						72.2	36	62514
						70.2	20	128781
						55.2	56	56754
						284.1	24	45717
						216.1	24	69693
						158	32	56847
						155	24	1526382
						144	44	41505
						130.1	52	41771
						127	52	1184444
						116	60	20368
						77.1	60	35078
						55.1	52	17162
						178.1	52	88416
						167.1	20	97389
						165.1	52	95726

					143.1	12	612375
					129	12	206428
					128	32	196308
					117.1	16	1235644
					115	40	353612
					91.1	32	2262178
					65.1	60	673049
2C-P	C13H21NO2	FIU_0174	8.83	224.2	207.1	8	2370806
					192.1	16	794252
					188.1	8	1326095
					163.1	28	393182
					149.1	28	102947
					135	24	155614
					105.1	36	205058
					103	48	116322
					91.1	56	235297
					79.1	52	176193
					77.1	60	382344

	2-methyl-a-Pyrrolidinopropiophenone	C14H19NO	FIU_0392	6.8	218.14666	160.1	8	1004880
						147.1	16	261616
						119.1	24	420128
						117.1	36	98367
						98.1	24	434430
						91.1	40	231079
						77.1	60	109283
						70.1	20	47186
						65.1	60	97081
						56.1	60	127391
						55.1	48	53439
	3-Methyl-a-Pyrrolidinobutiophenone (HCl)	C15H21NO	FIU_0135	7.26	232.2	174.1	8	2647797
						161.1	16	973302
						145.1	20	1275444
						133.1	20	426623
						119	28	581551
						112.1	28	829297

					105.1	24	2147103
					91.1	48	985537
					84.1	32	376516
					77.1	60	308484
					70.1	16	331020
					65.1	60	496869
4-acetoxy DMT	C14H18N2O2	FIU_0746	6.1	247.13683	202	12	38949
					160	20	75052
					159.1	4	465959
					142	40	4005
					134	20	2403
					132	36	12099
					131.5	40	1825
					117	48	11952
					115	56	30669
					105	52	3793
					58.1	16	184611
4-Fluoropentylindole	C13H16FN	FIU_0684	10.73	206.12668	186.1	12	5057
					141.2	56	98

					132.2	20	1970
					130	16	12745
					130	16	4013
					118.1	24	2631
					118	20	7318
					91.1	52	5001
					91	44	1834
					81.2	20	96
4-hydroxy DET	C14H20N2O	FIU_0747	5.8	233.15756	160	20	161985
					142	36	9052
					132	32	21432
					117	44	26820
					115	48	94958
					105	40	6792
					89	60	18928
					86.1	12	363422
					77	60	8781
					58.1	40	35609
4-methoxy PV9	C19H29NO2	FIU_0412	9.18	304.21983	233.2	16	431945

					168.2	28	615177
					135.1	36	258873
					121.1	28	901678
					107.1	52	67556
					84.2	44	178074
					77.1	60	300103
					72.2	36	40816
					69.2	60	64425
					55.2	56	72009
7-APB	C11H13NO	FIU_0424	7.02	176.09971	159.1	4	562444
					131.1	16	700125
					116.1	32	33120
					115.1	48	47814
					103.1	36	41825
					102.7	48	11973
					91.2	32	110412
					77.1	48	218512
					65.2	56	48948
					51.2	60	92417

ADB-PINACA N-(4-hydroxypentyl) metabolite	C19H28N4O3	FIU_0737	9.64	361.21614	358.1	4	72811
					231.1	20	89654
					213	32	124422
					185	40	5605
					175	36	11360
					171	44	5892
					145	52	54013
					90	60	1827
					69.1	48	44417
AM2201	C24H22FNO	FIU_0163	11.01	360.2	270.1	24	31572
					232.1	24	907050
					163	8	67680
					144	40	619525
					127	56	4584774
					116	60	269380
					105	40	38314
					89	60	44472
					77.1	60	128925

					69.1	40	44081
AM2201 7-hydroxyindole metabolite	C24H22FNO2	FIU_0631	10.95	376.16346	248.1	28	65361
					160	40	41201
					132.1	56	9005
					127	60	136036
					104	60	17978
					77.1	60	2802
					69.1	44	3169
AM2201 8-quinolinyl carboxamide	C23H22FN3O	FIU_0614	11.37	376.17469	232.1	16	302230
					212	40	2669
					199.6	44	53
					180.4	48	74
					171	8	2743
					158	40	2602
					155.1	24	4550430
					144	44	135298
					116	60	45282
					89	60	4037

					69.1	44	10497
AM2233	C22H23IN2O	FIU_0165	8.38	459.1	362	20	500976
					235.1	28	33379
					230.9	36	380866
					202.9	60	223095
					158	52	32211
					112.1	24	1649095
					98.1	40	2891913
					76.1	60	71428
					70.1	60	394360
					58.1	60	298433
Buphedrone metabolite ((±)- Ephedrine stereochemistry)	C11H17NO	FIU_0429	6.31	180.13101	162.1	8	825436
					133.1	20	183472
					132.1	32	76956
					131.1	16	87394
					104.1	36	33627
					91.1	32	190854
					79.1	36	28177

					77.1	60	54782
					70.1	20	31488
					65.1	60	59518
Desoxypipradrol (hydrochloride)	C18H21N	FIU_0182	8.17	252.2	167.1	20	428612
					165	52	204036
					152	48	156604
					131.1	20	505186
					129.1	28	306158
					128	52	213080
					117.1	24	176782
					115	48	281925
					91.1	36	1770614
					65.1	60	459065
JWH 018 6-hydroxyindole metabolite	C24H23NO2	FIU_0478	10.82	358.17288	230.1	24	67048
					221.7	0	103
					160	40	45661
					155	24	1157306
					132	60	21538

					127	60	885622
					105	60	6881
					77.1	60	20705
JWH 018 N-(3-methylbutyl) isomer	C24H23NO	FIU_0047	11.57	342.2	214.1	20	18863
					158.1	28	2081
					144	40	8185
					127.1	52	99556
					119.1	12	57
					116.1	60	3770
					77.1	60	6074
JWH 081 N-(4-hydroxypentyl) metabolite	C25H25NO3	FIU_0515	10.52	388.18344	230.1	24	77729
					185.1	24	1908398
					157.1	48	552662
					144	36	146817
					142	60	224647
					129	56	21513
					127	60	338286

					116	60	34358
					114	60	106689
					69.1	40	78971
JWH 146	C28H29NO	FIU_0524	12.28	396.22491	268.2	24	13823
					170	40	10554
					155	24	2750418
					127	60	1839506
					115.1	60	3990
					77.1	60	19670
					57.1	52	3327
JWH 167	C21H23NO	FIU_0526	11.16	306.17796	214.1	24	559569
					188.1	16	243544
					144	40	444672
					132.1	28	48261
					130	44	91819
					116	60	202160
					105	24	114231
					91.1	24	1346056
					77.1	56	78358

					65.1	60	130593
JWH 200 6-hydroxyindole metabolite	C25H24N2O3	FIU_0532	8.62	401.17869	155	24	461839
					127	60	235000
					114.1	32	134649
					100.1	60	3877
					86.2	56	3976
					84.1	56	13437
					70.1	56	37432
JWH 200 7-hydroxyindole metabolite	C25H24N2O3	FIU_0533	9.85	401.17869	351.1	16	103
					155	24	203327
					127	60	104923
					114.1	28	217002
					102.5	60	160
					100.1	44	5560
					86.1	60	4730
					84.1	56	14248
					70.1	56	40410
					56.1	60	3525

JWH 210 3-ethylnaphthyl isomer	C26H27NO	FIU_0071	11.99	370.2	214.1	24	2296544
					183.1	24	5064879
					155.1	40	2408535
					153.1	56	1421332
					144.1	44	1312734
					128.7	60	488880
					128.2	60	289546
					116.1	60	410274
					115.1	60	480211
					77.1	60	306644
LY2183240	C17H17N5O	FIU_0461	10.09	308.14331	280.2	0	310087
					192.2	16	4093
					167.1	16	106535
					165.1	60	12344
					152.1	56	8932
					87.1	12	30449
					72.2	32	146626
					59.2	24	5470
					56.1	60	4204

	MN-18	C23H23N3O	FIU_0728	11.78	358.18411	215	16	293262
						144.9	40	165572
						121.1	48	102
						116.9	60	20923
						90	60	34319
						89.5	60	11458
	Phenylpiracetam	C12H14N2O2	FIU_0766	7.51	219.10553	202	4	65359
						174	8	107141
						145	20	33287
						129	24	22618
						128	44	6897
						117	36	20935
						115	52	13111
						91	60	19074
						77	60	8740
						55.1	36	5891
	(S)-(+)-JWH 018 N-(4-hydroxypentyl) metabolite	C24H23NO2	FIU_0473	10.33	358.17288	340.2	16	10864
						284.2	24	15422

					230.1	24	11572
					186.2	12	19319
					155.1	20	837610
					144.1	40	31233
					127.1	56	568140
					116.1	60	11876
					77.2	60	9962
					69.2	40	23042
(S)-(+)-MT-45	C24H32N2	FIU_0325	9.61	349.25655	181.1	24	1348728
					179.1	40	109145
					169.2	16	481881
					167.2	16	110702
					166.1	40	463168
					165.1	60	376021
					153.1	40	54200
					103.1	56	452122
					87.2	32	143251
					77.1	60	148661
2,4,6-Trimethoxyamphetamine	C12H19NO3	FIU_0280	8.02	226.14	209.1	4	1206523

					181.1	20	500507
					151.1	24	65931
					136	32	83243
					121.1	28	176428
					93.1	52	54798
					91.1	40	144129
					78.1	60	80617
					77.1	60	110784
					65.1	60	104737
2-Amino-1-phenylbutane	C10H15N	FIU_0320	7.19	150.12045	133.1	4	310981
					105	8	1547
					91.1	16	654486
					65.1	44	215451
					63.1	60	20547
					51.1	60	19569
2C-G	C12H19NO2	FIU_0310	8.57	210.14158	193.1	4	344603
					178.1	12	658131
					163.1	28	284498
					133.1	20	32164

					115.1	52	35114
					105.1	40	50956
					91.1	48	113732
					79.1	44	47953
					77.1	60	62488
					65.1	60	44531
4-bromo-2,5-DMMA	C12H18BrNO2	FIU_0306	8.33	288.05209	257	12	408487
					229	20	204439
					199	32	65880
					178.1	20	215263
					163.1	32	97931
					135.1	40	117116
					105.1	48	114322
					91.1	60	72974
					79.1	60	51580
					77.1	60	104526
4-fluoromethcathinone (4-FMC)	C10H12FNO	FIU_0007	5.89	182.1	164.1	12	1980509
					149	20	1196810
					148	36	642220

					123	20	267291
					103	32	343195
					101	56	154930
					77.1	48	408254
					75.1	60	217329
					58.1	40	65859
					51.1	60	139634
5-methoxy DMT	C13H18N2O	FIU_0015	6.12	219.1	174.1	12	1425105
					159.1	28	435091
					143	32	189080
					131.1	40	347719
					130	52	600002
					115	52	79862
					103.1	60	155994
					78.1	60	80299
					77.1	60	138656
					58.1	12	2605704
AM251	C22H21Cl2IN4O	FIU_0166	11.85	555	454.9	32	1667176
					328	56	268291

					299	60	54521
					265	60	112885
					255.9	56	73088
					159.9	60	52877
					129	60	170832
					99.1	32	45102
					84.1	32	308062
					55.1	60	38288
AMB	C19H27N3O3	FIU_0744	11.23	346.20524	314.1	8	32421
					300.2	12	6456
					286.1	12	205903
					215.1	24	389298
					145	44	225686
					125.8	16	62
					117	56	27954
					90	60	48007
					89	60	16840
					89	12	29793
					71.1	40	2899

					55.1	20	41361
Diethylcathinone	C13H19NO	FIU_0347	6.32	206.14666	133.1	16	186937
					130.1	44	26890
					105.1	24	430105
					103.1	40	47096
					100.1	24	230351
					79.1	40	81874
					77.1	56	231588
					72.1	16	84231
					58.1	32	75245
					51.1	60	62423
JWH 073 6-hydroxyindole metabolite	C23H21NO2	FIU_0503	10.56	344.15723	216.1	24	98395
					213.3	32	107
					160.1	40	64696
					136.9	16	144
					132	60	33652
					127	56	1118593
					105	60	13126
					77.1	60	39530

					57.1	40	7292
JWH 203 3-chlorophenyl isomer	C21H22ClNO	FIU_0068	11.43	340.2	339.4	0	8250
					203.4	0	40
					124.9	24	1178
					75	56	93
					57.2	32	720
JWH 203 N-pentanoic acid metabolite	C21H20ClNO3	FIU_0537	10.12	370.11317	218.1	16	77909
					200.1	16	182016
					172.1	32	34683
					156	40	21682
					144	44	23472
					130	60	19993
					125	36	429943
					118	36	7164
					89.1	60	28460
					55.1	56	34506
JWH 210 2-ethylnaphthyl isomer	C26H27NO	FIU_0538	11.64	370.20926	214.1	24	841275

						183.1	24	1267260
						165.1	44	87040
						165.1	40	173014
						155.1	44	914973
						155.1	44	436246
						153.1	60	288961
						153.1	56	598823
						144.1	44	1089196
						144	44	540680
						141.1	40	623325
						141.1	36	299439
						128.7	60	204759
						128.7	60	103625
						116.1	60	382218
						115.6	60	260556
						115.6	60	177042
						115.2	60	126721
	JWH 210 N-pentanoic acid metabolite	C26H25NO3	FIU_0542	10.76	400.18344	244.1	24	38733
						183.1	28	400033

						155.1	44	107188
						153.9	60	36926
						144	40	36043
						129	60	31922
						127	60	14454
						115.1	60	28572
						83.1	40	9650
						55.1	56	25064
	RCS-4 N-(5-hydroxypentyl) metabolite	C21H23NO3	FIU_0576	14.53	338.16779	321.3	8	20744
						111.1	20	10109
						97.1	24	17556
						95.1	20	8011
						83.1	24	15315
						81.1	28	8002
						71.1	20	9805
						69.1	36	22008
						57.2	36	20356
						55.2	56	26679
11	2,4-Dimethylethcathinone	C13H19NO	FIU_0346	8.1	206.14666	188.2	8	795999

						173.1	20	242215
						165.1	8	1505695
						160.3	16	159358
						159.1	20	294710
						158.1	32	385094
						145.1	24	101115
						144.1	40	101386
						115.1	60	91814
						91.1	56	95586
						72.1	12	87081
	25I-NBOMe 3-methoxy isomer	C18H22INO3	FIU_0380	8.96	428.06444	291	20	270909
						276	32	99966
						272.1	16	223920
						174.1	8	2194709
						164.1	32	58691
						149.1	40	45034
						134.1	44	39398
						121.1	32	794681
						106.1	60	58169

					91.1	60	376311
					77.1	60	141918
2C-C	C10H14ClNO2	FIU_0143	7.54	216.1	199	8	987049
					184	16	465013
					174.1	8	14572
					169	32	260080
					164	20	96218
					103	28	42645
					91	48	130393
					78.1	60	60265
					77.1	48	221729
					65.1	60	70213
					51.1	60	67653
2C-H	C10H15NO2	FIU_0147	6.55	182.1	150	16	937820
					135	32	451049
					107	40	102394
					105.1	24	170097
					103	32	114571
					91.1	48	97059

					79.1	36	206042
					77.1	52	479482
					51.1	60	155428
2-Methyl-a-pyrrolidinobutiophenone (HCl)	C15H21NO	FIU_0121	7.2	232.2	161.1	16	712959
					133.1	20	303253
					119	28	751316
					112.1	24	1055298
					105.1	24	1401788
					91.1	48	1205154
					84.1	32	223744
					77.1	60	235736
					70.1	44	189210
					65.1	60	540930
3,4-Dimethylmethcathinone (HCl)	C12H17NO	FIU_0123	7.21	192.1	159.1	20	2665048
					158.1	36	1503972
					144	36	705768
					133.1	20	219496

					117.1	48	121135
					115	60	333069
					105.1	32	169940
					91.1	56	336569
					77.1	60	285566
4-Chloroamphetamine	C9H12ClN	FIU_0291	7.82	170.06583	153	4	158378
					125	16	207100
					99.1	48	20840
					90.9	52	2380
					90.1	44	21373
					89.1	48	46112
					75.1	48	3769
					73.1	60	11804
					65.1	52	5733
					63.1	60	24032
4-fluoro-a-Pyrrolidinopentiophenone	C15H20FNO	FIU_0398	7.44	250.15289	179.1	16	228898
					126.1	28	287573
					123	32	197197
					109.1	24	558343

					97.1	48	45289
					95.1	56	252583
					84.1	36	128980
					75.1	60	84285
					72.2	28	45165
					70.1	20	96416
4-Fluoromethcathinone metabolite ((±)-Ephedrine stereochemistry)	C10H14FNO	FIU_0354	6.19	184.10594	166.1	8	818656
					151.1	20	142427
					135.1	20	115570
					133.1	32	48524
					122	36	16291
					115.1	28	95728
					109.1	36	116852
					83.1	60	48972
					70.1	20	29709
					56.2	36	16915
4-methoxy DMT	C13H18N2O	FIU_0144	11.71	219.1	186.1	4	189442
					174.1	12	765906

					159	28	289512
					143.1	36	99279
					131	40	78350
					130	52	234200
					117	36	121096
					115	52	191205
					91.1	56	91946
					77.1	60	112737
					58.1	12	3365262
	4'-Methoxy-a-pyrrolidinopropiophenone (tosylate)	C14H19NO2	FIU_0145	6.53	234.1		
					163.1	16	1147358
					135.1	24	1348034
					105	36	503713
					103.1	48	248455
					98.1	24	1790794
					91.1	60	237389
					79.1	48	342539
					77.1	60	624919
					56.1	60	440154

					55.1	52	189835
4-methyl-a-Ethyltryptamine	C13H18N2	FIU_0751	7.98	203.147	146	8	15733
					144	16	161319
					130	48	8537
					129.2	44	6810
					128	56	6728
					115	56	20218
					91	52	15633
					77.1	60	8418
					58.1	20	16352
4'-Methyl-a-pyrrolidinohexanophenone (HCl)	C17H25NO	FIU_0149	8.49	260.2	324.1	12	63450
					253	20	67276
					189.1	16	1315960
					140.1	28	1054542
					119	28	892220
					105.1	24	2873232
					91.1	56	1121485
					84.1	40	487725

					77.1	60	344672
					72.1	20	243482
					70.1	20	210572
					65.1	60	421791
5-fluoro PB-22	C23H21FN2O2	FIU_0578	10.62	377.15871	232.1	12	2654377
					212.1	40	21801
					209.1	4	185715
					158.1	40	22853
					144	44	1192067
					130.1	52	19785
					116	60	443248
					89.1	60	42273
					69.1	44	91102
					67.2	60	8837
					61.2	56	15142
5-fluoro PB-22 N-(2-fluoropentyl) isomer	C23H21FN2O2	FIU_0590	10.82	377.15871	232.1	12	574530
					212	40	28610
					176	44	6320
					158	44	6128

					144	48	155674
					130	56	6148
					129.1	60	5787
					116	60	45782
					69.1	48	30593
					67.1	56	3916
5-methoxy DALT	C17H22N2O	FIU_0154	7.02	271.2	174.1	16	2100684
					159	36	696857
					143	40	289793
					131	44	497275
					130	60	904120
					115	60	115652
					110.1	12	3448027
					81.1	32	215470
					79.1	44	163195
					68.1	28	112500
6-IT	C11H14N2	FIU_0688	6.57	175.1157	158.1	4	196250
					143.1	28	12674
					130.1	20	80112

					117.1	24	38179
					115.1	52	14362
					103.1	40	9831
					91.1	40	5100
					90.1	52	10716
					89.1	60	13410
					77.1	52	25675
AB-FUBINACA isomer 1	C20H21FN4O2	FIU_0718	10.15	369.16485	352.1	4	58898
					109	52	60489
AM2201 benzimidazole analog	C23H21FN2O	FIU_0457	11.09	361.16379	273.1	24	140896
					233.1	20	226397
					177.1	28	382773
					155.1	32	692290
					145.1	32	201946
					129.1	48	116825
					127.1	60	852792
					117.1	56	41239
					102.1	60	40141
					90.1	60	36387

AM630	C23H25IN2O3	FIU_0167	10.37	505.1	135	24	1615419
					114.1	40	225440
					107	60	151319
					100.1	48	42491
					92	60	28435
					86.1	60	7886
					84.1	60	23231
					79.1	60	17900
					77.1	60	171294
					70.1	60	68974
AM694 N-pentanoic acid metabolite	C20H18INO3	FIU_0699	9.9	448.03314	244	36	7692
					234.1	40	11226
					230.9	24	414779
					220	28	11728
					202.9	60	173851
					200.1	12	12623
					144	56	10121
					104	60	24861
					76	60	69513

					55.1	60	13929
Buphedrone metabolite ((±)-Pseudoephedrine stereochemistry)	C11H17NO	FIU_0430	6.27	180.13101	162.1	8	1088855
					133.1	20	213663
					132.1	32	98325
					131.1	16	100686
					117.1	52	29999
					104.1	36	45698
					91.1	36	210167
					77.1	60	46459
					70.1	24	34837
					65.1	60	72759
CB-52	C26H43NO3	FIU_0178	11.95	418.3	361.2	12	442484
					224.2	12	122835
					125	24	161615
					123	44	184095
					83.1	32	69180
					81.1	40	48298
					69.1	44	60095

					67.1	60	62669
					58.1	20	2474757
					55.1	60	166212
Dimethocaine	C16H26N2O2	FIU_0432	6.27	279.19943	206.1	16	75459
					160.2	16	92041
					149	12	7976
					142.1	16	496900
					120	24	841008
					98.1	44	25077
					92.1	52	384112
					86.2	32	466953
					65.1	60	384141
					58.2	48	102919
DPT	C16H24N2	FIU_0755	7.42	245.19395	144	24	171262
					128	48	6590
					127	48	22985
					117	40	38226
					114.1	12	341500
					102.1	12	14512

					91	60	28506
					86.1	28	55626
					77	60	15700
					72.1	32	23049
Escaline	C12H19NO3	FIU_0318	6.99	226.13649	181.1	12	137130
					166.1	20	17023
					121.1	20	26900
					103.1	28	20788
					93.1	24	22839
					91.1	36	51623
					78.3	56	11686
					77.1	48	47579
					65.1	60	23350
Etizolam	C17H15ClN4S	FIU_0677	9.55	343.07059	314.1	24	73954
					289.1	24	11811
					279.1	28	5588
					259.1	40	13381
					224.1	48	11313
					223	52	9352

					210	48	6615
					206.1	24	11288
					191.1	52	1735
					138	40	12639
Eutylone (hydrochloride)	C13H17NO3	FIU_0185	6.7	236.1	218.1	12	1383223
					189.1	20	842136
					188.1	16	1843317
					174	36	756834
					160.1	24	400784
					145.1	40	250103
					116.6	48	279272
					116	56	167123
					86.1	16	323008
					65.1	60	374725
Hydroxy Bupropion	C13H18ClNO2	FIU_0761	7.58	256.10261	238.1	8	220908
					167	20	40913
					166.5	20	20295
					139	28	41290
					131	28	31026

						130	60	43493
						115	52	11580
						103	48	25437
						77	60	24691
						55.1	32	13209
JWH 018 benzimidazole analog	C23H22N2O	FIU_0481	11.69	343.17321	273.1	20		368881
					215.1	20		750681
					159.1	28		243050
					155	32		1345243
					155	24		1835622
					147	28		105809
					145	28		515310
					131.1	36		212214
					127	60		1789735
					117	52		107340
					90.1	60		96763
JWH 018 N-(4-oxo-pentyl) metabolite	C24H21NO2	FIU_0483	10.31	356.15723	272.1	20		60913
					228.1	16		29547

					211.6	8	90
					184	16	9683
					155	24	922434
					155	20	2588699
					144	36	61251
					127	56	1541042
					116	60	18891
					85.1	32	246131
					77.1	60	33814
JWH 019 N-(5-fluorohexyl) isomer	C25H24FNO	FIU_0492	11.09	374.18419	354.2	20	119050
					284.2	24	23901
					246.1	24	137610
					226.1	32	31390
					176	36	20211
					155	28	1411279
					144	48	103486
					127	60	1170744
					116	60	46287
					55.1	52	48828

JWH 030 2-naphthoyl isomer	C20H21NO	FIU_0497	11.52	292.16231	169.1	28	1262
					164.1	16	198288
					155	16	1700750
					136.1	24	18390
					127	48	1009673
					108	32	34589
					101.1	60	19416
					94	36	97720
					80.1	32	29778
					77.1	60	101277
					66.1	60	43408
JWH 073 5-hydroxyindole metabolite	C23H21NO2	FIU_0502	10.6	344.15723	310.7	4	45
					216.1	24	134220
					160	40	89532
					132	60	40736
					127	56	824013
					120.9	20	150
					105.1	60	5121

					77.1	60	26304
					57.2	44	9523
JWH 073 7-hydroxyindole metabolite	C23H21NO2	FIU_0505	10.85	344.15723	216.1	24	291460
					174.1	36	4426
					160	40	258390
					132	56	49055
					127	56	1651577
					104.1	60	102079
					101.1	60	9350
					77.1	60	62160
					57.1	40	19100
JWH 073 N-butanoic acid metabolite	C23H19NO3	FIU_0509	10.1	358.13649	230.1	20	36441
					212	24	12852
					155	24	682790
					144	40	41386
					127	52	478799
					116	60	12998
					87.1	36	22745

					77.1	60	10267
JWH 122 2-methylnaphthyl isomer	C25H25NO	FIU_0061	11.49	356.2	214.2	20	535
					141	44	996
JWH 122 7-methylnaphthyl isomer	C25H25NO	FIU_0065	11.72	356.2	141.1	44	1111
					115	60	630
JWH 122 N-(5-hydroxypentyl) metabolite	C25H25NO2	FIU_0521	10.61	372.18853	230.1	28	71118
					227.8	60	129
					223.4	56	144
					169.1	24	1345702
					144	44	94599
					141.1	52	735704
					140.4	52	41798
					115.1	60	263250
					69.2	44	26437
JWH 210 5-ethylnaphthyl isomer	C26H27NO	FIU_0072	11.96	370.2	214.2	24	1244992
					183.1	24	5912549

					155.1	40	2383227
					153.1	56	1658477
					144.1	44	786248
					128.7	60	611379
					128.2	60	363568
					116	60	259945
					115.1	60	649101
					77.1	60	294169
JWH 210 N-(5-hydroxypentyl) metabolite	C26H27NO2	FIU_0541	10.84	386.20418	230.1	28	84540
					183	24	1298232
					155.1	44	317176
					153.9	60	109044
					153	56	243491
					144	48	92372
					129	60	93591
					127.9	60	36917
					115	60	96031
					77.1	60	38162

	JWH 250 N-pentanoic acid metabolite	C22H23NO4	FIU_0547	9.93	366.16271	200.1	16	29494
						131.1	32	13369
						130.1	40	31165
						121.1	20	513783
						93.1	40	48991
						91.1	56	291466
						83.1	44	11566
						77.1	60	17423
						65.1	60	20063
						55.2	60	42411
	N-Phenylacetyl-L-prolylglycine ethyl ester	C17H22N2O4	FIU_0765	8.52	319.15796	216	4	280812
						201.1	12	6715
						188.1	12	265785
						91	60	84296
						70.1	24	409046
						65.1	60	6486
	PB-22 5-hydroxyisoquinoline isomer	C23H22N2O2	FIU_0601	11.3	359.16813	214.1	24	1875489

					174.1	40	117
					158.1	40	28091
					144	44	940990
					130.1	56	19928
					116	60	301147
					89.1	60	32696
					71.2	40	14989
					55.2	60	8188
PB-22 8-hydroxyisoquinoline isomer	C23H22N2O2	FIU_0607	11.3	359.16813	214.1	16	549358
					158	40	7238
					144	44	230136
					130	48	5274
					116	60	74609
					89	60	8750
					71.1	36	4206
					55.1	60	2421
PB-22 N-pentanoic acid metabolite	C23H20N2O4	FIU_0612	9.94	389.14231	244.1	12	1226669

					172.1	32	9977
					156.1	36	6109
					144	40	425898
					116	60	107190
					101.1	36	122565
					89.1	60	8807
					83.1	40	130835
					59.1	60	67042
					55.2	60	293916
PV8	C17H25NO	FIU_0413	8.47	260.19361	189.2	16	87330
					154.2	28	248551
					119.1	20	115271
					105.1	32	195068
					91.1	28	633622
					84.2	40	128743
					77.2	60	323753
					72.2	32	56424
					70.2	20	125628
					55.2	56	40688

RCS-4 3-methoxy isomer	C21H23NO2	FIU_0086	11.3	322.2	214.1	24	419753
					144	40	363539
					135	24	5293292
					116	60	165554
					107	36	2406541
					92	60	1221544
					79.1	48	222296
					77.1	60	3034318
					64.1	60	193370
					51.1	60	125214
Sildenafil	C22H30N6O4S	FIU_0768	8.69	475.20492	291.1	48	56
					283	44	2094
					100.1	32	6705
					99.6	32	4373
					58.1	52	16295
					56	52	1140
Sildenafil Citrate	C22H30N6O4S	FIU_0769	8.69	475.20492	283.1	44	1906
					100.1	32	6317
					99.6	28	3824

					70.1	56	999
					58.1	48	15123
					56.1	56	1181
URB937	C20H22N2O4	FIU_0273	9.56	355.2	230	8	330117
					213	24	363074
					187	28	59223
					185	36	41119
					169	40	40962
					157	48	60945
					141	52	77129
					139	56	21273
					128	60	48593
					115	60	52598
XLR11 Degradant	C21H28FNO	FIU_0660	11.09	330.21549	232.1	24	247404
					216.8	40	53
					167.1	56	179
					144	40	117723
					130.1	56	2416
					116.1	60	50927

						89.1	60	9664
						83.1	32	4543
						69.2	40	10825
						55.2	56	8715
	XLR11 N-(4-hydroxypentyl) metabolite	C21H28FNO2	FIU_0664	10.42	346.21041	347.3	0	201476
						248.7	20	66194
						248.1	20	1230020
						210	8	292584
						144	36	829514
						116	60	238449
						87.1	36	121677
						67.1	48	205737
						59.1	60	137686
						57.1	44	49883
						55.1	56	105980
12	(-)-CP 55,940	C24H40O3	FIU_0091	11.68	377.3	301.2	4	43476
						233.2	12	58611
						216.1	20	131
						175.1	4	61840

					71.1	20	30646
(±)-JWH 018 N-(3-hydroxypentyl) metabolite	C24H23NO2	FIU_0470	10.58	358.17288	284.1	24	47167
					230.2	24	32699
					174.1	8	720367
					158.1	32	34602
					155.1	24	965041
					144.1	44	19174
					141.1	32	7420
					130.1	52	23549
					127.1	56	691464
					77.2	60	14360
					59.2	48	7184
1'-Naphthoyl-2-methylindole	C20H15NO	FIU_0681	10.3	286.11536	158.1	20	61936
					130.1	44	16449
					128	56	2163
					127	40	151965
					121.1	16	1677157
					103.1	56	9937

					101	60	4656
					77.1	60	34676
2,3-Dimethylmethcathinone	C12H17NO	FIU_0348	7.58	192.13101	159.1	20	666939
					158.1	32	340794
					144.1	36	181175
					116.8	52	20047
					115.1	56	85202
					105.1	32	32381
					91.1	56	76106
					77.1	60	69453
					58.2	24	28737
25N-NBOMe	C18H22N2O5	FIU_0383	7.98	347.15287	93.1	36	200830
					91.1	52	1330738
					77.1	60	34996
					65.1	60	187609
2C-N	C10H14N2O4	FIU_0155	6.55	227.1	151	16	65265
					137	16	27450
					107	28	14687
					105.1	36	19878

					103.1	40	26554
					91	44	66672
					79.1	40	27742
					77.1	56	93303
					65.1	60	60175
2-Ethylamino-1-phenylbutane	C12H19N	FIU_0321	7.43	178.15175	133.1	8	169511
					105.1	12	3808
					91.1	16	925276
					65.1	56	309318
					63.1	60	24781
					51.1	60	16920
2-Ethylmethcathinone (hydrochloride)	C12H17NO	FIU_0113	7.21	192.1	146.1	16	736520
					145.1	20	1376349
					144.1	36	1432023
					131	28	239164
					130	44	140197
					128	48	139152
					103.1	56	127973

					77.1	60	414584
					58.1	28	71419
2-Fluoroamphetamine (hydrochloride)	C9H12FN	FIU_0114	6.21	154.1	137	4	924895
					109	16	1881739
					89.1	40	46503
					83.1	44	604590
					81.1	48	16666
					75.1	60	13315
					65.1	52	15318
					63.1	56	122396
					59.1	48	94382
					57.1	60	304545
3,4-Dimethylmethcathinone metabolite ((±)-Ephedrine stereochemistry)	C12H19NO	FIU_0350	7.78	194.14666	176.1	8	669818
					161.1	20	99275
					145.1	20	56679
					130.1	28	47662
					129.1	36	33898

					115.1	56	31667
					105.1	32	58958
					91.1	48	27896
					77.1	60	34026
					56.1	28	41907
3,4-Dimethylmethcathinone metabolite ((±)- Pseudoephedrine stereochemistry)	C12H19NO	FIU_0351	7.74	194.14666	176.1	8	910702
					174.1	8	715283
					161.1	24	135654
					145.7	28	55552
					145.1	20	75197
					130.1	28	59990
					129.1	40	45849
					115.1	52	44416
					105.1	32	67546
					91.1	52	41421
					56.2	32	57003

3-Ethylmethcathinone (HCl)	C13H19NO	FIU_0127	7.33	206.2	178.1	8	491172
					146	16	4561
					145.1	24	8362
					144.1	36	10491
					131.1	24	835
					77	60	2458
3-Fluoroamphetamine (HCl)	C13H19NO	FIU_0128	6.28	206.2	134.8	20	115
3-Iodoamphetamine	C9H12IN	FIU_0288	8.39	262.00144	245	8	116842
					216.9	16	164042
					213	8	2310
					183.2	20	1651
					118.1	20	41960
					117.1	40	87754
					115.1	60	29667
					91	60	44327
					90.1	40	114642
					89.1	60	93152
3-methoxy PCP	C18H27NO	FIU_0386	8.67	274.20926	189.1	12	476961

					121.1	28	592000
					91.1	56	255444
					86.1	8	1047493
					81.1	24	173567
					79.1	52	45930
					78.1	60	100685
					77.1	60	115538
					69.2	56	26960
					65.1	60	94306
4-Bromoamphetamine	C9H12BrN	FIU_0290	8.1	214.01531	348.1	4	27217
					320.1	12	30764
					249	24	28269
					197	8	79688
					169	16	86136
					119.5	20	98
					118.1	20	25826
					117.1	36	37896
					115.1	60	11525
					91.1	60	20024

					90.1	44	31054
					89.1	60	27467
					64.2	60	2641
4-Fluorobuphedrone	C11H14FNO	FIU_0426	6.54	196.10594	150.1	16	179807
					149.1	24	299614
					148	40	165491
					147	16	1620514
					135	32	41709
					119.1	24	2148167
					109.1	28	137909
					101.1	60	44622
					95.1	48	58274
					83.1	56	46606
					75.1	60	89841
4'-Methyl-a-pyrrolidinopropiophenone (HCl)	C14H19NO	FIU_0150	6.83	218.2	117	36	365629
					98.1	28	1597591
					91	44	988360
					77.1	60	434401

					70.1	20	255411
					65.1	60	368092
					56.1	56	518796
					55.1	48	215520
4-Methylbuphedrone	C12H17NO	FIU_0427	7.15	192.13101	161.1	8	186293
					159.1	16	93287
					146.1	16	183068
					145.1	20	356441
					144.1	36	365359
					105.1	24	158574
					91.1	44	76908
					77.1	60	120838
					65.1	60	73948
4-methylethcathinone (4-MEC)	C12H17NO	FIU_0009	7.03	192.1	174.1	8	2536175
					146.1	16	1030649
					145.1	20	1207627
					144.1	32	1201326
					131.1	28	462798
					130.1	44	381702

					119.1	24	386789
					115	56	209152
					91.1	40	577057
					77.1	60	376686
5-fluoro AB-PINACA N-(4-hydroxypentyl) metabolite	C18H25FN4O3	FIU_0705	8.93	365.19107	222.3	40	71
					174.9	40	2447
					155.1	28	4845629
					152.5	32	68
					144.9	48	16562
					67.1	60	3763
5-fluoro PB-22 4-hydroxyisoquinoline isomer	C23H21FN2O2	FIU_0581	10.8	377.15871	232.1	20	289686
					212	40	2916
					200	28	45
					180.6	60	61
					158	40	2486
					144	44	137719
					116	60	47185

					89	60	3930
					69.1	48	9809
					61.1	60	1736
5-fluoro PB-22 6-hydroxyisoquinoline isomer	C23H21FN2O2	FIU_0585	10.44	377.15871	232	20	282512
					212.1	40	3133
					158	40	2264
					144	48	132682
					141.8	60	124
					116	60	50425
					89	60	4592
					69.1	44	10149
					61.1	56	1616
5-fluoro PB-22 N-(4-fluoropentyl) isomer	C23H21FN2O2	FIU_0592	10.64	377.15871	352	4	64567
					324.1	12	61697
					253	24	59264
					232.1	12	459513
					212.1	36	36483

					176	40	4295
					158	48	4630
					144	44	160238
					130	56	2890
					116	60	57799
					89	60	6760
					69.1	48	27476
					61.1	60	2220
5-Fluoropentyl-3-pyridinoylindole	C19H19FN2O	FIU_0685	9.89	311.14814	235.1	24	11835
					232.2	32	17185
					223.1	28	10462
					205.1	36	5103
					194.1	36	9538
					144.1	44	36103
					116.1	60	22055
					106	28	12614
					80.1	44	14298
					78.1	48	9683
AB-FUBINACA isomer 2	C20H21FN4O2	FIU_0719	9.94	369.16485	109	48	59051

Acetildenafil	C25H34N6O3	FIU_0771	8.53	467.26924	297.1	48	24767
					166.1	60	25303
					127.1	36	46811
					112.2	36	28000
					111	32	66049
					97.1	60	22972
					84.1	48	36287
					72.1	48	32613
					70.1	60	34349
					56.1	60	11976
ADBICA N-(4-hydroxypentyl) metabolite	C20H29N3O3	FIU_0695	9.63	360.22089	343.2	4	584215
					315.2	12	5344
					257.1	8	8459
					230.1	20	602090
					144	36	280196
					116	60	57897
					104.1	4	3889
					89	60	6065

					87.1	32	8109
					69.1	44	185100
ADBICA N-pentanoic acid metabolite	C20H27N3O4	FIU_0697	9.52	373.20016	357.1	8	322095
					329.1	12	7203
					244	20	326262
					172.1	40	2222
					144	40	116045
					116	60	26602
					101	40	30463
					83	44	31052
					59.1	60	19717
					55.1	60	94469
AM1241	C22H22IN3O3	FIU_0161	8.69	504.1	407	20	119512
					275.9	40	66098
					229.9	60	53848
					112.1	28	417591
					98.1	28	3132890
					84.1	60	18049
					81.1	56	18914

					70.1	60	306760
					58.1	60	151378
					55.1	60	21745
AM2201 N-(3-fluoropentyl) isomer	C24H22FNO	FIU_0029	11.23	360.2	232.1	24	894009
					212.1	36	101562
					163.1	8	34633
					155.1	28	5352422
					144	44	332788
					127.1	56	4564750
					116.1	60	153770
					105	44	20278
					77.1	60	115583
					69.1	48	160157
AM2201 N-(4-fluoropentyl) isomer	C24H22FNO	FIU_0030	11.02	360.2	340.2	20	120647
					284.1	24	83465
					232.1	24	851798
					212.1	36	127795
					144	44	492945

					127.1	60	4729212
					116.1	60	235998
					77.1	60	112815
					69.1	44	99980
AM2201 N-(4-hydroxypentyl) metabolite	C24H22FNO2	FIU_0632	10.15	376.16346	248.1	24	10447
					155	28	170129
					144	40	15849
					127	60	123105
					116	60	3892
					87.1	40	1643
					67.1	48	3631
					59.1	60	2061
AM2232	C24H20N2O	FIU_0164	10.24	353.2	225.1	24	539640
					155	24	718606
					155	20	4192055
					144	48	339634
					129	56	20337
					127	56	3291319

					116	60	230106
					89	60	47582
					82.1	40	24923
					77.1	60	98507
					55.1	60	92567
deschloro-N-ethyl-Ketamine	C14H19NO	FIU_0297	6.97	218.14666	173.1	8	551118
					145.1	16	432062
					131.1	28	32303
					129.1	24	34373
					117.1	32	58630
					115.1	52	55787
					91.1	36	767639
					77.1	60	75845
					67.1	20	98397
					65.1	60	184741
Diethylcathinone (hydrochloride)	C13H19NO	FIU_0183	6.08	206.2	155	24	2056130
					133	16	946456
					130	40	143864

					105.1	24	2274253
					103.1	40	246885
					100.1	24	1396183
					79.1	40	410062
					77.1	56	1202677
					72.1	16	462075
					58.1	32	401950
					51.1	60	340274
JWH 018 2'-naphthyl-N-(1,2-dimethylpropyl) isomer	C24H23NO	FIU_0036	11.63	342.2	272.1	20	201855
					214.1	20	266516
					155	24	3598355
					144	36	1076583
					127.1	56	2466159
					116	60	420088
					101	60	9991
					89.1	60	82543
					77.1	60	55947
					71.1	32	50244

	JWH 018 5-hydroxyindole metabolite	C24H23NO2	FIU_0477	10.86	358.17288	230.1	24	97003
						174.1	36	3958
						160	40	65046
						135.2	24	115
						132	56	21901
						127	56	609279
						77.1	60	16508
	JWH 018 7-hydroxyindole metabolite	C24H23NO2	FIU_0479	11.08	358.17288	230.6	24	1551
						230.1	24	289567
						160	40	279592
						132	56	39869
						127.1	44	2016
						127	56	1842672
	JWH 018 8-quinoliny carboxamide	C23H23N3O	FIU_0480	12	358.18411	340.2	12	5172
						214.1	12	2643200

						171	8	26241
						158.1	40	35645
						144	40	1171077
						130.1	48	20114
						116	60	360281
						89.1	60	47453
						71.2	40	19174
						55.1	60	10564
JWH 018 N-(1,2-dimethylpropyl) isomer	C24H23NO	FIU_0042	11.43	342.2		272.1	20	383059
						254.1	36	13295
						214.1	20	291346
						155.1	24	4168628
						155	24	1402048
						144	36	1180414
						127.1	56	3084598
						116	60	431380
						89.1	60	90547
						77.1	60	69772
						71.1	32	68986

JWH 019 N-(2-fluorohexyl) isomer	C25H24FNO	FIU_0489	11.31	374.18419	246.1	24	368044
					226.1	40	22010
					169.1	52	1322
					155	28	1900413
					144	44	135109
					127	60	1593275
					116	60	45671
					77.1	60	19828
					61.1	60	12212
					55.1	52	61164
JWH 019 N-(6-fluorohexyl) isomer	C25H24FNO	FIU_0494	11.11	374.18419	246.1	24	132803
					155	28	893384
					144	44	87211
					127	56	763832
					116	60	33645
					94.8	40	102
					61.1	52	6728
					55.2	56	20777

JWH 073 N-(2-hydroxybutyl) metabolite	C23H21NO2	FIU_0506	10.37	344.15723	284.1	24	17758
					216.1	24	183504
					144	40	130902
					127	52	1204520
					116	60	50957
					89.1	60	10544
					77.1	60	36919
					73.1	36	17045
					55.1	48	36990
JWH 073 N-(2-methylpropyl) isomer	C23H21NO	FIU_0054	11.28	328.2	155	20	1902
JWH 073 N-(4-hydroxybutyl) metabolite	C23H21NO2	FIU_0507	10.1	344.15723	216.1	24	21941
					205.2	8	105
					204.2	60	108
					155	24	651464
					144.1	40	38558
					127	56	471078
					116.1	60	16213

					77.1	60	12306
					55.1	48	18474
JWH 122 5-methylnaphthyl isomer	C25H25NO	FIU_0063	11.73	356.2	169	24	1385
					141	40	1351
					115.1	60	870
JWH 145 2-phenyl isomer	C26H25NO	FIU_0523	11.48	368.19361	319.1	4	7246
					287	20	3852
					257	28	3209
					240.2	20	45902
					227	40	3335
					196.9	52	3168
					170	40	40575
					155	20	2204135
					127	52	1544608
					77.1	60	27336
JWH 200 2'-naphthyl isomer	C25H24N2O2	FIU_0067	9.52	385.2	298.2	16	4292
					221	12	69
					155.1	20	195990

					127	60	90687
					114.1	28	72068
					84.1	56	5913
					70.1	60	18366
JWH 210 6-ethylnaphthyl isomer	C26H27NO	FIU_0073	12.01	370.2	214.2	24	1953525
					183.1	28	5373899
					155.1	44	4112413
					144	44	1022318
					140.1	60	1196513
					129.1	60	365496
					128.2	60	216570
					116.1	60	329063
					115.6	60	294456
					77.1	60	126829
JWH 210 7-ethylnaphthyl isomer	C26H27NO	FIU_0074	11.92	370.2	214.1	24	1498231
					183.1	24	6486207
					155.1	44	4399405
					144.1	44	835153

					140.1	60	1438861
					128.7	60	420728
					128.2	60	239839
					116	60	274094
					115.1	60	337574
					77.1	60	136041
JWH 398 7-chloronaphthyl isomer	C24H22ClNO	FIU_0082	11.83	376.2	270	28	4304
					214.1	24	422784
					189	24	5322561
					161	52	4131989
					158	36	7402
					144	40	273263
					130	56	8230
					126	60	1097324
					116	60	92140
					89.1	60	13907
JWH 398 N-pentanoic acid metabolite	C24H20ClNO3	FIU_0553	10.78	406.11317	275.9	60	84
					247.1	40	101

					189	28	88924
					161	56	56748
					126	60	7275
					55.2	56	2961
Mephedrone metabolite ((±)- Ephedrine stereochemistry)	C11H17NO	FIU_0338	6.99	180.13101	162.1	8	768126
					147.1	20	144546
					131.1	20	100460
					116.1	28	40766
					115.1	48	49576
					105.1	32	59998
					91.1	28	115468
					77.1	60	38070
					65.1	60	31402
					56.1	28	28849
MN-25-2-methyl derivative	C27H39N3O3	FIU_0446	10.6	454.29914	275.2	20	551216
					190.2	36	30146
					188.2	40	26043
					137.2	36	24367

					114.2	36	987061
					95.2	60	49812
					84.2	60	41499
					81.2	56	144164
					70.2	60	118484
					67.2	60	14254
N,N-DMA	C11H17N	FIU_0309	6.32	164.1361	119.1	8	239934
					91.1	24	845533
					77	60	7650
					72.2	12	5534
					65.1	52	258856
					63.1	60	20164
					51.1	60	22385
N-ethyl-N-Methylcathinone	C12H17NO	FIU_0335	6.13	192.13101	133.1	16	225851
					130.1	44	12605
					105.1	24	428456
					103.1	40	49636
					86.1	24	253295
					79.1	40	82229

					77.1	52	217680
					60.2	16	17139
					58.2	44	115131
					51.1	60	78471
Pyrazolam	C16H12BrN5	FIU_0680	8.33	354.02761	285.1	24	4575
					281.2	0	65
					249.5	20	82
					228.3	60	69
					206.1	36	13632
					205.1	52	10745
					174.1	8	3356067
					167.1	36	14923
					155	60	2083
					115.9	60	319
					78.1	60	3592
Thiosildenafil	C22H30N6O3S2	FIU_0770	9.86	491.18208	341.1	32	10456
					327.1	32	5788
					313.1	40	4000
					299.1	44	10563

						155.1	20	174405
						100.1	32	14555
						99.6	32	10758
						85.1	36	4206
						70.1	60	4116
						58.1	56	45026
						56.1	60	4768
	XLR11 N-(4-pentenyl) analog	C21H27NO	FIU_0665	11.53	310.20926	311.3	0	209681
						212.1	20	411479
						144	40	198994
						130	48	95769
						125.1	20	1601517
						97.1	28	320808
						83.1	20	206324
						69.1	36	232328
						57.2	48	345974
						55.1	44	676564
13	(-)-11-nor-9-carboxy-Δ9-THC	C21H28O4	FIU_0089	11.54	345.2	327.2	12	45436

					299.2	20	26249	
					193.1	28	11488	
					187.1	32	5960	
					123	44	5074	
					119	32	6641	
					105	52	3620	
					91.1	60	6413	
					79.1	48	4070	
					69.1	44	5538	
	1,4-Dibenzylpiperazine (HCl)	C18H22N2	FIU_0106	7.51	267.2	188.2	8	620951
						176.2	16	93571
						175.1	16	1221085
						146.1	20	69539
						134.1	24	738685
					120.1	20	139521	
					118.1	60	46551	
					104.1	32	69276	
					91.1	36	4171244	
					65.1	60	914480	

					56.1	32	40208
2-Bromoamphetamine	C9H12BrN	FIU_0281	7.76	214.01531	197	8	93000
					169	16	178864
					118.1	20	7878
					117.1	36	17700
					115.1	60	9729
					91.2	56	14736
					90.1	44	77102
					89.1	60	67356
					64.2	60	7315
					63.1	60	9157
2-Chloroamphetamine	C9H12ClN	FIU_0282	7.52	170.01	153.1	8	156899
					125	16	309933
					115.1	44	4499
					99.1	48	31493
					91.3	44	6609
					90.1	48	33007
					89.1	44	71313
					73.1	60	20150

					65.2	56	15159
					63.1	60	35294
3-Chloroamphetamine	C9H12ClN	FIU_0287	7.76	170.06583	174.1	8	2550771
					153	4	101647
					125	16	231341
					105.3	20	102
					99	44	20837
					90.1	48	22077
					89.1	44	44883
					75.1	48	3633
					73.1	60	12654
					65.1	48	5414
					63.1	60	24892
3'-fluoro-a-Pyrrolidinopropiophenone	C13H16FNO	FIU_0394	6.17	222.12159	178.1	12	1206753
					151	16	129977
					123	24	349783
					103.1	36	227859
					98.1	28	513166

					95.1	52	94417
					84.1	36	60404
					77.1	56	184576
					70.1	20	194135
					56.1	52	147388
					55.1	52	67358
3-hydroxy Phenazepam	C15H10BrClN2O2	FIU_0673	9.56	364.96142	258.1	28	3609
					257.1	36	3255
					213	44	3091
					210	24	1553
					208.2	36	1214
					206.1	40	7718
					179.1	56	4059
					178.1	60	4398
					176.1	8	1413195
					151	60	3279
3-Methylethcathinone (hydrochloride)	C12H17NO	FIU_0133	6.77	192.1	146.1	16	1081702
					144.1	32	1271568
					131	28	517821

					130	44	457786
					119.1	24	360883
					91.1	40	541974
					77.1	60	387880
					65.1	60	300296
4-CAB	C10H14ClN	FIU_0322	8.31	184.08148	188.1	8	2305526
					167	4	106482
					125	12	285411
					107.1	20	85
					99	48	28500
					90	48	26570
					89.1	52	56607
					75.1	52	4645
					73	60	15538
					65.1	56	5701
					63.1	60	30559
4-Ethylmethcathinone (hydrochloride)	C12H17NO	FIU_0137	7.33	192.1	159.1	20	186543
					146.1	16	762999
					145.1	20	1530849

					144.1	36	1563277
					131.1	28	206958
					130	44	120557
					105.1	28	245244
					103.1	52	145054
					77.1	60	487937
4-hydroxy DiPT	C16H24N2O	FIU_0748	6.48	261.18886	160.1	20	1230835
					142.1	40	60745
					135.1	4	233520
					132.1	36	164998
					117	48	167989
					114.1	12	555085
					105.1	52	48410
					102.1	12	87423
					89.1	60	51912
					77.1	60	36219
					72.2	32	210003
4-methyl-N-Methylbuphedrone	C13H19NO	FIU_0428	7.12	206.14666	161.1	12	505408
					133.1	16	158480

					119	24	174087
					105.1	20	562910
					91.1	40	260995
					86.1	24	242605
					77.1	60	87857
					71.1	48	108300
					65.1	60	190940
					56.1	60	79852
5-APB (hydrochloride)	C11H13NO	FIU_0152	7.01	176.1	132.1	16	66475
					131	16	1378255
					116	32	99438
					115	48	135102
					103	40	68392
					91.1	32	374196
					77.1	48	416424
					65.1	60	144923
					51.1	60	163102
5-EAPB	C13H17NO	FIU_0419	7.18	204.13101	159.1	8	708956
					131.1	20	900722

					129.1	28	23444
					116.1	36	49139
					115.1	56	79483
					103.1	44	45986
					91.1	40	198594
					77.2	56	259075
					65.2	60	76006
					51.2	60	54056
5-fluoro ABICA	C19H26FN3O2	FIU_0703	9.88	348.20091	331.1	4	1129723
					304.1	12	222680
					232.1	20	1433917
					212.1	36	11555
					158	40	10870
					144	44	645384
					130	56	10884
					116	60	251772
					89	60	33780
					69.1	44	52556
					61.1	60	9220

5-fluoro PB-22 3-hydroxyquinoline isomer	C23H21FN2O2	FIU_0580	11.02	377.15871	232.1	20	1882439
					212.1	40	17483
					176.1	40	11809
					158.1	44	16479
					144	44	929363
					130.1	56	14975
					116	60	319989
					89.1	60	30929
					69.1	44	69993
					61.1	60	10396
5-fluoro PB-22 4-hydroxyquinoline isomer	C23H21FN2O2	FIU_0582	10.87	377.15871	232.1	8	1897728
					212.1	40	17054
					176	40	9731
					158	40	15619
					144	44	888120
					130	52	12541
					116	60	324045
					89.1	60	34703

					69.1	44	74062
					61.1	52	9706
5-fluoro PB-22 5-hydroxyquinoline isomer	C23H21FN2O2	FIU_0584	10.76	377.15871	232.1	20	1809130
					212.1	44	16787
					176	44	10839
					158	40	17693
					144	48	976533
					130	60	15052
					116	60	334482
					89.1	60	30601
					69.1	44	76819
					61.1	60	12076
5-fluoro SDB-005	C23H21FN2O2	FIU_0439	11.23	377.15871	233.1	8	1251693
					213.1	28	292691
					185.1	32	22057
					177.1	32	110835
					171.1	36	28304
					145.1	44	448148
					121.1	48	16250

					117	60	79467
					90.1	60	122458
					69.2	36	154775
5-Fluoropentylindole	C13H16FN	FIU_0686	10.72	206.12668	233.1	20	236953
					142.2	12	92
					137.6	16	44
					132.1	16	3395
					132	16	12878
					118	20	21692
					118	20	5697
					91.1	48	2746
					91.1	44	11067
					69.1	20	3575
					65.1	60	3595
5-IT	C11H14N2	FIU_0687	5.89	175.1157	158.1	4	233062
					143.1	28	14453
					130.1	20	98165
					117.1	24	46424
					115.1	52	14682

					103.1	40	12302
					91.1	44	5086
					90.1	52	12945
					89.1	60	16807
					77.1	56	31415
5-MAPDB	C12H17NO	FIU_0421	6.33	192.13101	366.1	4	55817
					338.1	12	62083
					161.1	8	960213
					133.1	24	635520
					117.1	48	27725
					115.1	56	36630
					105.1	36	103195
					103.1	44	59521
					91.1	60	65140
					79.2	40	89541
					77.1	60	188927
					51.2	60	42113
AB-PINACA N-(4-hydroxypentyl) metabolite	C18H26N4O3	FIU_0724	9.2	347.20049	284.1	20	4799

					231	20	25948
					213	28	45745
					174.9	32	3039
					171	44	2165
					144.9	48	17703
					102.8	40	66
					69.1	44	14503
ADB-FUBINACA	C21H23FN4O2	FIU_0732	10.44	383.1805	253	24	53397
					225	40	712
					211.2	28	70
					154.6	36	66
					109	56	53668
ADBICA N-(5-hydroxypentyl) metabolite	C20H29N3O3	FIU_0696	9.63	360.22089	343.1	8	599813
					315.1	12	9915
					230.1	20	690910
					144	44	293888
					130	52	3493
					116	60	74818

					89	60	7235
					87.1	36	4688
					69.1	44	96537
					67.1	56	4104
ADB-PINACA pentanoic acid metabolite	C19H26N4O4	FIU_0739	9.51	375.19541	330.1	12	69957
					284.1	16	737394
					245	24	30480
					227	36	23476
					217.1	32	39583
					199	48	5377
					185	48	3804
					175	52	6631
					145	60	11949
					55.1	60	17335
AH 7921	C16H22Cl2N2O	FIU_0671	8.51	329.11092	202	20	100510
					190	24	195101
					173	32	641453
					145	60	293644
					109	60	47317

					95.2	32	369619
					93.1	56	31944
					67.2	60	206759
					55.2	60	109266
AKB48	C21H31N3O	FIU_0158	12.94	342.3	(blank)	(blank)	(blank)
AM1220 azepane isomer	C26H26N2O	FIU_0627	8.9	383.20451	286.1	20	15779
					155	28	125473
					127	60	131479
					112.1	20	839474
					98.1	28	97887
					84.1	56	18887
					81.1	56	14939
					70.1	60	61167
					58.1	60	215786
					55.2	60	25027
AM1248	C26H34N2O	FIU_0162	9.73	391.3	155	24	4618817
					135.1	32	3376107
					112.1	36	1318693
					107.1	60	263159

					98.1	52	1078622
					93.1	60	391400
					81.1	60	112984
					79.1	60	324495
					70.1	60	231906
					67.1	60	119298
					58.1	60	396787
AM2201 5-hydroxyindole metabolite	C24H22FNO2	FIU_0629	10.26	376.16346	248.1	24	17710
					160	44	12361
					155	28	115739
					132	60	5614
					127	56	97682
AMT	C5H10N2S	FIU_0757	3.98	131.05647	124.5	16	41
					112	12	83
					104	12	2698
					77	16	8437
					72	20	9163
					60	52	25194
					53.1	32	1650

	a- Pyrrolidinopentiothiophenone	C13H19NOS	FIU_0408	6.59	238.11873	167.1	12	156888
						126.2	20	810428
						111.1	40	109663
						97.1	24	336963
						84.2	40	124674
						83.6	56	42469
						70.2	16	46047
						69.2	60	108807
						55.8	56	37649
						55.2	48	65152
	Benzedrone	C17H19NO	FIU_0343	8.71	254.14666	236.1	8	142434
						162.1	12	11638
						146.1	12	63950
						144.1	12	8925
						131.1	24	6432
						119.1	24	13124
						91.1	20	1209268
						65.1	60	345750

					63.1	60	13133
					51.1	60	6527
bk-DMBDB (hydrochloride)	C13H17NO3	FIU_0170	6.46	236.1	191.1	12	1389419
					163	16	638643
					161	16	1482770
					149	24	1017098
					121	40	496318
					105.1	32	494796
					86.1	24	2124511
					77.1	60	372129
					71.1	52	677383
					65.1	56	1016942
CMP	C10H17N	FIU_0303	7.03	152.1361	155	24	4289659
					121.1	8	52041
					93.1	12	84915
					91.1	28	23268
					79.1	20	289089
					77.1	36	262848
					65.1	56	8519

					58.2	8	489652
					55.1	24	13917
					53.1	56	13086
					51.1	60	132741
Diclozepam	C16H12Cl2N2O	FIU_0676	9.98	319.03267	256.1	24	1554
					227.1	32	13124
					205.1	44	3994
					165.1	60	5018
					155	24	2894192
					154	32	14542
					125	56	5629
					118.1	60	2725
					117.1	48	3278
					91.1	52	2821
					58.1	36	3030
FDU-PB-22	C26H18FNO2	FIU_0593	11.43	396.13216	224.1	28	6092
					109	40	723015
					106.4	56	98
					83.1	60	20354

	Harmaline	C13H14N2O	FIU_0186	7.26	215.1	200.1	24	1124468
						174.1	24	1154314
						172.1	32	1180156
						171.1	44	808670
						159	32	267578
						157.1	44	217445
						143.1	44	143862
						131.1	44	491377
						130	56	501056
						68.1	24	277321
	HU-210	C25H38O3	FIU_0187	12.14	387.3	261.1	12	44036
						243.1	16	96186
						201.1	24	35670
						147	20	10741
						133.1	24	16865
						123	28	10340
						105.1	56	10650
						85.1	24	72769
						71.1	24	158553

					57.1	28	104560
JW 618	C17H14F6N2O2	FIU_0443	10.13	393.09595	373.1	36	24136
					197.1	44	126781
					183.8	48	7457
					183.1	48	60025
					181.1	60	14660
					170.3	44	24900
					169.1	44	686760
					155.1	60	67743
					154.1	60	110466
					141.1	60	12087
JWH 018 2'-naphthyl-N-(2-methylbutyl) isomer	C24H23NO	FIU_0039	11.73	342.2	214.1	24	551061
					155	20	38635
					144	40	555355
					130.1	56	6344
					127	56	3485598
					116	60	211698
					101.1	60	18963
					89.1	60	41824

					77.1	60	106649
					71.1	36	46570
JWH 018 2'-naphthyl-N-(3-methylbutyl) isomer	C24H23NO	FIU_0040	11.74	342.2	214.1	24	458478
					158.1	36	50092
					144	40	247990
					130.1	48	30177
					127	56	2328641
					116	60	101936
					89.1	60	19311
					77.1	60	79788
					71.2	36	14399
JWH 018 N-(1-ethylpropyl) isomer	C24H23NO	FIU_0482	11.28	342.17796	272.1	20	206130
					272.1	20	83125
					254.1	40	13422
					254.1	40	5639
					214.1	20	402340
					214.1	20	152939
					155.1	24	381314

					155	24	2155201
					144	36	1298335
					144	36	558080
					127	56	1658579
					116	60	551772
					116	60	236169
					89.1	60	125729
					89.1	60	49949
					77.1	60	98264
					77.1	60	39847
					71.1	32	19865
					71.1	32	6198
JWH 018 N-(2-methylbutyl) isomer	C24H23NO	FIU_0046	11.53	342.2	214.2	20	5474
					155	24	1606321
					144.1	36	5198
					127	56	34781
JWH 019 N-(6-hydroxyhexyl) metabolite	C25H25NO2	FIU_0495	10.53	372.18853	244.1	28	35101
					238.9	48	198

					207.9	44	125
					155	20	1245939
					144	48	40651
					127	60	860916
					116.1	60	17289
					77.1	60	9426
					55.1	56	32369
JWH 071	C21H17NO	FIU_0499	10.67	300.13101	172.1	20	426098
					157.1	40	10195
					144	44	134235
					129	56	22301
					127.1	48	1476597
					116	60	115658
					101.1	60	29869
					89.1	60	48266
					77.1	60	154180
JWH 081 4-hydroxynaphthyl metabolite	C24H23NO2	FIU_0514	11.11	358.17288	214.1	24	269636
					188.1	20	40299

					171	24	1642061
					144.1	40	162541
					143	48	618568
					132.1	32	10449
					118	40	5594
					115.9	60	67210
					115	60	743543
					89.1	60	22114
JWH 122 N-(4-hydroxypentyl) metabolite	C25H25NO2	FIU_0520	10.65	372.18853	354.2	16	17483
					298.1	24	29841
					230.1	24	45751
					229.1	4	37350
					212.1	28	10644
					173.1	8	8802
					169.1	24	1823193
					144	40	106982
					141.1	52	1027290
					115.9	60	32254

					115.1	60	340781
					85.2	16	25633
					69.1	40	66305
JWH 133	C22H32O	FIU_0522	11.11	313.24532	205.1	4	1250
					181.2	48	123
					149	8	2097
					91	48	4136
JWH 147	C27H27NO	FIU_0218	12.18	382.2	333	8	3008
					254.1	20	34243
					226.9	40	1340
					170	44	29961
					155	20	5029637
					142	52	8601
					127	56	3626908
					115.1	60	12122
					101	60	11221
					77.1	60	60434
JWH 149	C26H27NO	FIU_0525	11.83	370.20926	243.4	48	107
					228.1	24	468716

						169.1	28	2673026
						158.1	44	283943
						141.1	52	1804444
						130.1	60	92031
						115	60	658829
						103.1	60	22656
						91	60	11309
	JWH 203 N-(4-hydroxypentyl) metabolite	C21H22ClNO2	FIU_0535	10.26	356.13391	338.2	16	25992
						282	20	30445
						204.1	16	42172
						186.1	16	430745
						170.1	48	17383
						144.1	44	23626
						130.1	24	79701
						125	32	619379
						89.1	60	51486
						69.1	40	35670

	JWH 210 N-(4-hydroxypentyl) metabolite	C26H27NO2	FIU_0540	10.87	386.20418	230.1	24	63232
						183.1	24	1892839
						155.1	44	486182
						153	56	393758
						144	40	136724
						129.1	60	150146
						128.2	60	59583
						115	60	150287
						77.1	60	56364
						69.1	44	77445
	JWH 398 3-chloronaphthyl isomer	C24H22ClNO	FIU_0079	11.96	376.2	214.1	24	262038
						189	28	2917443
						161	52	2289818
						158	36	5018
						144	44	196136
						130	56	5222
						126	60	601245

					116	60	65883
					89	60	9554
JWH 398 5-chloronaphthyl isomer	C24H22ClNO	FIU_0080	11.97	376.2	270.1	36	3702
					214.1	24	361956
					189	24	4063660
					161	48	3199653
					158.1	36	7012
					144	44	254584
					130	48	7288
					126	60	756038
					116	60	83352
					89.1	60	12689
JWH 398 8-chloronaphthyl isomer	C24H22ClNO	FIU_0083	11.31	376.2	340.2	24	38331
					270.1	36	34681
					214.1	28	238945
					189	28	4586007
					161	52	3373594
					146.1	8	1429267

					144	40	211135
					131	20	816522
					130.1	56	7816
					126	60	925642
					116	60	77163
					89.1	60	12016
MDAI (hydrochloride)	C10H11NO2	FIU_0243	11.782	178.1	131	20	277158
					103	32	354760
					77.1	52	293751
					51.1	60	106209
Methedrone	C11H15NO2	FIU_0010	6.32	194.1	176.1	8	2593400
					161.1	20	1379942
					146	32	858946
					145.5	28	246777
					135.1	20	242611
					118.1	44	454903
					91.1	56	391925
					79.1	40	173533
					77.1	56	417317

					58.1	12	471655
MN-25	C26H37N3O3	FIU_0445	10.2	440.28349	353.3	28	78423
					261.2	24	267191
					217.1	36	48978
					176.1	36	71369
					174.1	40	47701
					137.2	36	39758
					114.1	40	504852
					95.2	52	66498
					81.2	60	186610
					70.2	60	68648
Naphyrone 1-naphthyl isomer	C19H23NO	FIU_0084	8.31	282.2	211.1	16	634288
					169	24	145025
					155	28	490281
					141.1	28	776045
					126.1	24	769882
					115	60	200937
					84.1	48	180376
					70.1	20	65982

					55.1	60	76564
N-methyl-2-Al	C10H13N	FIU_0317	5.98	148.1048	117.1	16	745779
					115.1	32	329754
					91.1	36	304108
					89.1	56	34377
					77.1	48	11083
					75.1	60	4363
					65.1	56	153680
					63.1	60	37007
					51.1	60	30733
Nor-Mephedrone (hydrochloride)	C10H13NO	FIU_0254	6.61	164.1	147.1	8	201264
					130	36	687326
					119	16	207010
					103	52	110042
					91.1	36	150947
					77.1	60	301147
					65.1	56	103942
					51.1	60	81411

PB-22 6-hydroxyquinoline isomer	C23H22N2O2	FIU_0604	11.31	359.16813	214.1	24	411246
					158	40	6410
					144	44	199712
					130	56	4401
					126.7	60	65
					116	60	61074
					89	60	6384
					71.1	44	3062
Pentedrone (hydrochloride)	C12H17NO	FIU_0256	7.057	192.1	161.1	8	510846
					144	32	430191
					132.1	16	772372
					130	40	459333
					117	32	336023
					91.1	28	1014627
					77.1	60	656771
					65.1	60	301127
					51.1	60	266623
UR-144 Degradant N-pentanoic acid metabolite	C21H27NO3	FIU_0646	10.48	342.19909	244.1	20	57216

						176.5	16	68
						144	36	23063
						116.1	60	8985
						101.1	36	6142
						83.1	36	7757
						59.1	56	4559
						55.1	56	23410
	Yangonin	C15H14O4	FIU_0465	9.95	259.08921	231.2	12	78628
						171.1	24	49168
						161.1	20	137944
						139.1	60	36375
						133.1	36	66535
						128.1	52	41466
						115.1	60	24714
						90.1	60	25123
						77.1	60	33963
						69.1	48	37128
	14 (±)-CP 47,497	C21H34O2	FIU_0097	11.67	319.3	301.2	4	24031
						233.1	12	28527

						226.9	28	1494
						197.1	40	1428
						133	52	5222
						121	32	11495
						107	24	14007
						85.2	16	8095
						77.1	60	6563
						71.1	20	14290
						57.1	36	10199
	(±)-JWH 018 N-(2-hydroxypentyl) metabolite	C24H23NO2	FIU_0469	10.63	358.17288	284.1	28	21416
						254.1	60	4621
						230.1	24	101702
						144.1	44	91478
						127.1	56	817309
						116.1	60	35208
						89.1	60	4593
					77.1	60	17714	
					69.2	40	5432	

2,3-Dimethylethcathinone	C13H19NO	FIU_0345	7.83	206.14666	173.1	20	232208
					163.1	8	558776
					160.2	16	148676
					158.8	20	268980
					158.1	32	367489
					145.1	24	98906
					144.1	36	101749
					115.1	60	89230
					91.1	56	85830
					77.1	60	71684
2,3-methylenedioxymethcathinone	C11H13NO3	FIU_0109	11.65	208.1	190.1	8	430283
					160	12	2882810
					147	16	279464
					132.1	28	1466222
					117	40	633380
					91.1	40	420629
					77.1	56	222660
					65.1	60	497039

					58.1	36	115650
2-Fluoroethcathinone (hydrochloride)	C11H14FNO	FIU_0115	11.66	196.1	178.1	12	1092910
					151.1	8	199124
					149.7	16	546160
					149.6	20	457069
					148	36	353858
					135	32	279012
					123.1	16	183850
					123	24	209992
					115	32	89959
					103	32	228837
					77.1	52	320695
					75.1	60	135381
2-methylmethcathinone	C11H15NO	FIU_0120	11.66	178.1	151.1	8	211745
					145.1	20	722045
					144.1	32	511387
					130.1	32	55545
					119	20	74660
					103	48	55344

					91.1	40	107801
					77.1	60	166297
					65.1	56	73074
					58.1	32	31495
3,4-DHMA	C10H15NO2	FIU_0305	4.27	182.11028	133.1	20	18576
					123	20	151527
					105.1	24	42300
					103	36	11144
					79.1	32	21121
					77.1	44	76833
					65.1	44	7688
					58.2	8	5874
					51.1	60	58179
3-Fluoroethcathinone (HCl)	C11H14FNO	FIU_0129	11.65	196.1	150	16	892141
					149.6	20	467320
					135	32	410172
					123	24	203519
					108	52	95938
					103	32	252893

					95	48	95259
					77.1	52	320637
					75	60	149438
3-Fluoromethamphetamine (hydrochloride)	C10H14FN	FIU_0130	11.65	168.1	137	8	805811
					109	20	2680962
					89	48	76510
					83.1	48	969544
					81	60	16842
					75.1	60	16683
					63.1	60	197604
					59.1	52	143123
					58.1	12	97914
					57.1	60	398762
4-MTA	C10H15NS	FIU_0294	7.76	182.09252	165.1	4	462929
					159.1	4	1653592
					137	20	126330
					137	20	126152
					122	40	45522

					122	36	43631
					121	56	38656
					121	52	32766
					118.2	20	38467
					118.1	20	39402
					117.1	16	154489
					117.1	16	152897
					115.1	48	55193
					115.1	44	55333
					91.1	56	84746
					91.1	52	89665
					78.1	56	23063
					78.1	52	25419
					65.1	60	32902
					65.1	60	31975
5-APDB	C11H15NO	FIU_0418	6.35	178.11536	161.1	8	533807
					133.1	20	324650
					115.1	48	18506
					105.1	32	52434

					103.1	40	28649
					91.1	52	33821
					79.2	40	46977
					77.1	52	93973
					65.2	60	17851
					51.2	60	31531
5-fluoro ADB-PINACA	C19H27FN4O2	FIU_0707	10.31	363.2118	233.1	24	129299
					213	36	36452
					177	40	16380
					171	48	3884
					145	52	68238
					117	60	7985
					90	60	8331
					69.1	44	19585
5-fluoro MN-18	C23H22FN3O	FIU_0709	11.19	376.17469	233	16	292485
					213	28	81304
					185	32	5540
					177	32	28956
					171	40	7161

					144.9	44	121557
					117	60	18824
					100	12	440
					90	60	27952
					69.1	36	37153
5-fluoro PB-22 6-hydroxyquinoline isomer	C23H21FN2O2	FIU_0586	10.7	377.15871	232.1	24	1724282
					212.1	44	17773
					176	44	12447
					158.1	40	18859
					144	48	1028711
					130.1	56	15546
					116	60	348729
					89.1	60	32038
					69.1	44	76768
					61.1	60	11638
5-fluoro-AKB48 N-(4-hydroxypentyl) metabolite	C23H30FN3O2	FIU_0710	11.14	400.23221	135.1	20	406809
					107	56	37201

					93.1	60	50742
					91	60	7116
					81.1	60	14845
					79.1	60	45566
					77.1	60	8398
					69.1	60	4909
					67.1	60	18195
					55.1	60	6684
AB-CHMINACA	C20H28N4O2	FIU_0714	10.98	357.22123	352.1	4	83982
					340.1	4	104201
					324.1	12	94885
					312.1	12	98202
					253	28	82248
					241	28	115482
					144.9	48	69296
					116.9	60	4689
					97	44	7288
					90	60	1171
					69.1	56	3621

					55.1	60	35886
AB-PINACA N-(4-fluoropentyl) isomer	C18H25FN4O2	FIU_0723	9.99	349.19615	332.1	4	58625
					330.1	4	46751
					302.1	12	54078
					284.1	16	5199
					233	24	64428
					213	36	16674
					177	40	5870
					145	48	27110
					117	60	4245
					90	60	6314
					69.1	44	10836
ADB-PINACA isomer 2	C19H28N4O2	FIU_0735	10.88	345.22123	328.1	4	207429
					300.1	12	247452
					232.1	20	15493
					215.1	24	286784
					145	48	167539
					117	60	17974
					90	60	21948

					71.1	48	2332
AKB48 N-(4-hydroxypentyl) metabolite	C23H31N3O2	FIU_0741	11.31	382.24163	135	24	310963
					107	56	27265
					93	60	43090
					91	60	7644
					81.1	60	12013
					79	60	35986
					77	60	7698
					69.1	60	4118
					67.1	60	15021
					55.1	60	6471
AM2201 2'-naphthyl isomer	C24H22FNO	FIU_0027	11.17	360.2	232.1	24	463477
					163	8	9926
					144	44	330566
					127	56	2216269
					116	60	146060
					105.1	40	5199
					89	60	24115

					77.1	60	53409
					69.1	44	27086
AM2201 6-hydroxyindole metabolite	C24H22FNO2	FIU_0630	10.27	376.16346	248.1	24	14665
					160	44	7817
					155.1	24	173975
					155	28	148864
					132	60	4293
					127	56	144520
					114.6	28	61
					77.1	60	2585
AM694	C20H19FINO	FIU_0169	10.66	436.1	309.1	20	515345
					292.1	32	119869
					234.1	32	327306
					232.1	36	206578
					230.9	28	3116193
					202.9	56	1565046
					144	56	150562
					104.9	60	89457
					104	60	216463

					76.1	60	805764
AM694 3-iodo isomer	C20H19FINO	FIU_0031	11.24	436.1	232.2	32	48782
					230.9	28	2194949
					202.9	52	1261167
					144	48	53916
					130	60	10259
					116	60	17989
					105.2	56	11374
					104	60	67662
					76.1	60	680805
					69.1	48	4334
BB-22	C25H24N2O2	FIU_0615	11.48	385.18378	386.4	0	7263
					241.1	8	5833
					240.1	12	2845950
					158.1	40	10635
					144	44	1073268
					116	60	180879
					97.1	40	147856
					89.1	60	12550

					69.1	52	82062
					55.1	56	780505
BB-22 3-hydroxyquinoline isomer	C25H24N2O2	FIU_0617	11.95	385.18378	317.9	0	49
					240.1	20	503971
					158	40	2615
					144	44	228525
					116	60	33823
					97.1	40	29854
					89	60	2531
					69.1	52	16693
					55.1	60	159037
BB-22 4-hydroxyisoquinoline isomer	C25H24N2O2	FIU_0618	11.74	385.18378	240.1	20	590644
					158	40	2578
					144	44	249444
					116	60	37491
					97.1	40	32649
					89	60	2815

					69.1	52	18503
					55.1	60	178525
BB-22 7-hydroxyisoquinoline isomer	C25H24N2O2	FIU_0624	11.56	385.18378	253.4	4	63
					240.2	24	280690
					156.4	60	65
					144	44	141992
					116	60	22255
					97.1	40	19553
					69.1	52	11222
					55.1	60	114145
bk-MDDMA (hydrochloride)	C12H15NO3	FIU_0171	11.75	222.1	177	12	497035
					149	20	540536
					147	20	1146862
					119	28	445349
					91.1	40	956948
					72.1	20	2079145
					70.1	52	111698
					65.1	60	793312

					58.1	32	79344
					57.1	56	82914
Cannabigerol	C21H32O2	FIU_0467	11.27	317.24023	193.2	16	105095
					137.1	40	4064
					123.1	36	29015
					95.1	48	5148
					81.1	52	4998
					79.1	60	3657
					77.1	60	3774
					69.2	28	3690
					67.2	60	9192
					55.2	60	7308
CB-25	C25H41NO3	FIU_0177	11.71	404.3	387.3	12	346061
					347.3	16	630695
					287.2	4	5282
					221.1	12	224307
					181.1	24	670223
					175.1	4	6168
					111	40	196515

					83.1	28	92248
					71.1	36	113438
					69.1	40	94246
					58.1	24	2605479
					55.1	56	215424
HU-211	C25H38O3	FIU_0188	12.12	387.3	261.1	12	49403
					243.1	16	129988
					201.1	24	42624
					147	20	11084
					133.1	24	17500
					105.1	52	12872
					95.1	20	13170
					85.1	24	91828
					71.1	24	175373
					57.1	36	117221
HU-311	C21H28O3	FIU_0190	11.73	329.2	314.2	12	11011
JW 642	C21H20F6N2O3	FIU_0444	10.87	463.13781	183.1	28	1162802
					168.1	60	138467
					165.1	56	137652

					155.1	48	129893
					155	24	4095481
					153.9	60	64958
					129.1	60	59785
					127.7	60	24063
					127.1	60	32187
					115.1	60	49317
					77.1	60	34529
JWH 016	C24H23NO	FIU_0196	11.439	342.2	214.1	24	531989
					158	40	317344
					155	24	1909915
					130.1	56	113829
					127	56	3720079
					103.1	60	42839
					101	60	26994
					77.1	60	154113
					57.1	44	31665
					51	60	6351

JWH 018 2'-naphthyl-N-(1,1-dimethylpropyl) isomer	C24H23NO	FIU_0035	11.59	342.2	144	36	1885405
					120.7	16	101
					116	60	691057
					101.1	60	5201
					89.1	60	136667
					77.1	60	27431
					71.1	32	60124
JWH 018 2'-naphthyl-N-(2,2-dimethylpropyl) isomer	C24H23NO	FIU_0038	11.65	342.2	272.1	24	20828
					214.1	24	512814
					155.1	28	581873
					144	36	652737
					127	56	3266340
					116	60	231221
					101	60	16327
					89.1	60	41230
					77.1	60	89445
					71.1	36	59576

JWH 018 N-(1-ethylpropyl) isomer	C24H23NO	FIU_0043	11.39	342.2	155	24	4929262
					127	56	3847552
JWH 018 N-(2,2-dimethylpropyl) isomer	C24H23NO	FIU_0045	11.43	342.2	214.2	24	4490
					155	24	108778
					144	32	6790
					127.1	56	36813
					116.1	60	2330
					77	60	1577
JWH 073 4-hydroxyindole metabolite	C23H21NO2	FIU_0501	11.25	344.15723	216.1	24	170177
					160	40	110347
					132.1	48	20566
					127	60	399774
					104.1	60	53011
					77.1	60	14907
					57.2	44	11146
JWH 073 4-methylnaphthyl analog	C24H23NO	FIU_0212	11.61	342.2	200.1	24	694343
					169	24	2523920

						158	36	12256
						155	24	1365619
						144	40	411867
						141	48	1687110
						116.1	60	154476
						115	60	923228
						91.1	60	22355
						89.1	60	32394
						57.1	44	48839
JWH 081 3-methoxynaphthyl	C25H25NO2	FIU_0056	11.66	372.2		214.1	24	1640497
						185	24	3247995
						157.6	36	29087
						157	36	153891
						144	40	989738
						130.2	52	25575
						129	44	1328825
						128	60	1353255
						127.6	60	1083946
						116	60	331907

	JWH 081 5-methoxynaphthyl	C25H25NO2	FIU_0057	11.69	372.2	214.1	28	635460
						185.1	24	5748473
						185	24	2122887
						170	44	76497
						157	40	1304054
						144	44	490156
						142	60	69563
						129	48	885170
						128.5	60	1190426
						127	60	2758699
						116	60	181210
	JWH 081 8-methoxynaphthyl	C25H25NO2	FIU_0060	11.36	372.2	185	16	5080778
						170	48	2856931
						155	48	60649
						142	60	241953
						141	40	112625
						129	44	226009
						127.7	60	206652

					127	60	592046
					115.1	60	54893
					114	60	437505
JWH 116	C26H27NO	FIU_0519	11.81	370.20926	242.2	24	222632
					172.1	40	128704
					157	56	14423
					155	28	2809704
					144.1	56	31195
					129.1	60	20172
					127.1	60	2328859
					124.6	56	150
					117.1	60	10398
					77.1	60	34622
JWH 210 2-ethylnaphthyl isomer	C26H27NO	FIU_0070	11.67	370.2	214.1	24	1724013
					183.1	24	2635884
JWH 210 8-ethylnaphthyl isomer	C26H27NO	FIU_0075	11.68	370.2	214.2	24	590695
					183.1	24	2452270
					165.1	36	407856

					155.1	44	1493220
					153.1	60	385078
					144.1	44	365853
					141.1	44	110114
					140.1	60	448160
					128.7	60	135692
					127.1	60	145653
JWH 307 5'-isomer	C26H24FNO	FIU_0548	11.33	386.18419	258.1	24	62268
					258.1	24	33273
					188.1	40	33942
					188	40	54009
					160	56	13896
					155.1	20	1703780
					140.9	44	111
					133	60	29733
					133	60	10846
					127.1	60	1223076
					101	60	18979
					77.1	60	104656

					77.1	60	17375
					75.1	60	3554
					51.1	60	5986
JWH 398 N-(4-hydroxypentyl) metabolite	C24H22ClNO2	FIU_0551	10.9	392.13391	374.1	16	10095
					318.1	28	14989
					254.2	56	4877
					243.2	60	109
					189	24	660972
					186.1	12	20003
					161	56	407534
					144.1	44	12916
					126	60	61783
					69.1	40	14061
LY2183240 2'-isomer	C17H17N5O	FIU_0462	10.38	308.14331	192.1	16	5876
					167.1	16	141147
					165.1	60	16540
					152.1	56	12037
					87.1	12	42396

					72.2	36	183659
					59.2	20	7404
					56.1	60	5844
MAM2201 N-(4-hydroxypentyl) metabolite	C25H24FNO2	FIU_0640	10.45	390.17911	248.1	24	19106
					169.1	24	171349
					144	40	23459
					141.1	52	97499
					117.6	44	67
					115	60	27474
					87.1	40	2348
					67.1	44	4512
					59.1	60	2865
MAM2201 N-(5-chloropentyl) analog-d5	C25H19D5ClNO	FIU_0642	11.38	395.18603	253.1	28	25431
					252	28	13307
					170	32	35394
					169.1	28	122216
					149.1	48	12245

					148.1	48	6692
					142.2	56	19661
					141.1	56	88035
					115.1	60	22900
					69.2	44	8188
MDA 77	C21H23N3O3	FIU_0242	11.78	366.2	355	8	41
					337.1	12	85104
					207.3	48	110
					175	20	26462
					161	8	456167
					119	16	21653
					105	20	5186463
					77.1	60	3741867
					51.1	60	161475
MDMA methylene homolog (hydrochloride)	C12H17NO2	FIU_0244	7.19	208.1	180	16	1054437
					177	12	241215
					147.1	16	237407
					135	20	1816798
					119.1	20	142294

					105	40	108255
					91.1	36	184690
					79.1	36	185885
					77.1	48	798463
					55.1	28	79055
					51.1	60	625342
Methylphenidate	C14H19NO2	FIU_0758	7.15	234.14158	174.1	24	4008
					134.6	12	56
					129	40	3527
					128	56	3188
					115	56	2897
					91	60	7695
					84.1	16	370067
					67.1	56	5769
					65.1	60	2959
					56.1	56	78057
NM2201	C24H22FNO2	FIU_0635	11.35	376.16346	232.1	8	165033
					179.7	20	66
					144	44	63532

					116	60	23804
					89.1	60	2949
					69.1	44	6403
NNEI 2'-naphthyl isomer	C24H24N2O	FIU_0448	11.35	357.18886	214.2	20	549904
					188.2	12	12488
					158.1	40	8189
					144.1	44	250574
					132.1	32	2227
					130.1	48	5579
					116.1	60	81782
					89.2	60	8571
					71.2	44	4211
					55.1	60	2750
Norsufentanil	C16H24N2O2	FIU_0255	7.841	277.2	245.1	8	319177
					128.1	8	1196540
					96.1	20	1657435
					94.1	40	150409
					81.1	48	172662
					80.1	60	118942

						77.1	60	98955
						69.2	40	84199
						68.1	48	108879
						67.1	44	260507
	PB-22 4-hydroxyisoquinoline isomer	C23H22N2O2	FIU_0599	11.39	359.16813	214.1	16	2225963
						158	36	29863
						144	40	964732
						130.1	48	16363
						116	60	319712
						89.1	60	35401
						71.2	40	16900
						55.1	60	9593
15	(-)-CP 47,497	C21H34O2	FIU_0090	11.67	319.3	133	52	5390
						121	32	7826
						107	24	13752
						85.1	12	6450
						77.1	60	3998
						71.1	12	13494

					57.1	20	9928
(+)-CP 55,940	C24H40O3	FIU_0093	11.68	377.3	211.3	12	104
					201.8	48	111
					149.1	12	2256
(±)3-epi CP 47,497-C8-homolog	C22H36O2	FIU_0559	12.38	333.27153	257	20	1476
					227	28	1550
					199.3	44	106
					196.9	40	1519
					167	60	1339
					124.3	20	93
					71.2	12	5630
					57.2	20	5896
(±)5-epi CP 55,940	C24H40O3	FIU_0096	11.67	377.3	359.3	0	14939
					233.1	4	6903
					219.3	8	119
					215.1	12	6483
					158.2	60	147
					121.1	20	4504
					93.1	36	2085

					71.1	20	3807
					57.2	28	2824
(±)-JWH 018 N-(4-hydroxypentyl) metabolite	C24H23NO2	FIU_0471	10.34	358.17288	340.2	16	13207
					284.2	24	19432
					230.1	24	14344
					186.2	12	24746
					155.1	20	1042394
					144.1	40	36994
					127.1	56	705941
					116.1	60	13978
					77.1	60	11690
					69.2	36	28266
1-(4-Fluorobenzyl) piperazine (HCl)	C11H15FN2	FIU_0103	11.65	195.1	175.1	12	136158
					109	20	1787937
					89	52	53117
					85.1	12	182619
					83.1	56	684570

					81.1	60	7712
					63.1	60	113071
					59.1	60	95812
					57.1	60	187981
					56.1	28	70798
2-AI (HCl)	C9H11N	FIU_0111	11.66	134.1	117	12	691352
					115.1	24	263781
					91.1	32	250680
					89.1	48	26626
					77.1	44	11406
					75.1	60	3814
					74	60	3350
					65.1	52	129437
					63.1	60	36873
					51.1	60	33380
3,4-Dimethoxymethamphetamine (HCl)	C12H19NO2	FIU_0122	11.66	210.1	179.1	8	3108697
					164.1	20	239844
					151	20	1215109

					138.1	20	181366	
					136	24	122021	
					121	32	150430	
					107	40	274328	
					91.1	40	295370	
					77.1	60	591332	
					65.1	60	167026	
	3',4'-Methylenedioxy-a-pyrrolidinobutiophenone (HCl)	C15H19NO3	FIU_0124	6.65	262.2	191	16	1194804
					188.1	8	2267557	
					163.1	20	689317	
					161	20	1498141	
					149	32	785534	
					133	28	416705	
					121	48	394286	
					112.1	28	1697208	
					105.1	40	485438	
					84.1	36	317090	
					65.1	60	847141	

3-Ethylethcathinone (HCl)	C13H19NO	FIU_0126	11.65	206.2	160.1	16	940937
					159.1	20	1472649
					144.1	32	1749084
					131.7	20	382214
					130	44	353936
					117	36	179114
					105.1	32	347628
					91.1	56	208802
					77.1	60	519681
3-Methoxymethcathinone (hydrochloride)	C11H15NO2	FIU_0132	11.65	194.1	161.1	20	959356
					146	28	456167
					145.6	24	156949
					133.1	28	127138
					132.1	36	248485
					118	40	212382
					91.1	56	226803
					79.1	36	73823
					77.1	56	218110
4-APB	C11H13NO	FIU_0416	7.13	176.09971	131.1	16	408457

					129.1	24	14969
					116.1	32	22590
					115.1	48	38812
					103.1	36	18883
					91.1	32	96922
					77.2	48	109322
					65.2	60	40052
					51.2	60	43175
4-Bromomethcathinone	C10H12BrNO	FIU_0353	7.52	242.01023	145.1	16	467160
					144.1	40	289388
					132.1	20	29385
					131.1	48	19805
					128.1	60	21744
					104.1	40	19868
					103.1	56	31340
					78.2	60	25106
					77.1	60	46914
					58.2	60	8656
4-FA (4-fluoroamphetamine)	C9H12FN	FIU_0139	6.26	154.1	137	4	762528

						109	16	986151
						101.1	48	4093
						89.1	40	25518
						83	44	314664
						81	52	5921
						75.1	60	13231
						63.1	60	67353
						59.1	48	51592
						57.1	60	154673
	4-Fluoromethamphetamine (hydrochloride)	C10H14FN	FIU_0142	11.69	168.1	137	8	1278139
						109	20	2314861
						101	56	6381
						89.1	48	63120
						83	52	735154
						81	60	11987
						75.1	60	20924
						63.1	60	151872
						59.1	56	113028

					57.1	60	313974
4-Hydroxyamphetamine	C9H13NO	FIU_0292	7.82	152.09971	110.3	32	99
					107.1	16	189502
					91.1	44	7007
					79.1	32	18793
					77.1	40	76831
					65.1	60	8147
					55.1	48	8408
					53.2	52	4617
					51.1	56	28604
5-fluoro PB-22 7-hydroxyquinoline isomer	C23H21FN2O2	FIU_0588	10.66	377.15871	232.1	20	399667
					223.1	20	55
					212	40	3831
					158	40	3726
					144	44	187525
					130	56	3272
					116	60	63500
					89	60	6519
					69.1	48	13519

					61.1	60	2532
5-fluoro-THJ	C22H21FN4O	FIU_0711	11.56	377.16994	359.1	20	99056
					213.1	28	89616
					177	36	38771
					171	40	11608
					145	44	160772
					117	60	27113
					90	60	34529
					89.2	60	14289
					69.1	36	44434
5-IAI (hydrochloride)	C9H10IN	FIU_0153	11.72	260	242.9	12	124229
					159.5	48	91
					154.8	16	105
					116	28	327950
					115	56	262746
					89	60	10210
A-834735 degradant	C22H29NO2	FIU_0690	10.9	340.21983	242.1	20	1383090
					144	44	121338
					125	24	38316

					99.1	32	508813
					83.1	32	31822
					81.1	44	156458
					79.1	60	52332
					69.1	48	301286
					57.1	44	115133
					55.1	56	117964
AB-FUBINACA 2-fluorobenzyl isomer	C20H21FN4O2	FIU_0716	10.26	369.16485	109	60	74428
ADBICA	C20H29N3O2	FIU_0694	10.89	344.22598	327.1	4	294416
					299.2	12	8506
					214.1	20	366724
					158	40	4458
					144	44	136230
					130	52	3416
					121.1	28	74
					116	60	44077
					89	60	5325
AM1235	C24H21FN2O3	FIU_0160	11.16	405.2	277.1	24	436955
					231.1	40	42921

					189	36	189810
					172	56	12444
					155	28	694010
					144.1	56	8474
					143	56	208191
					127	60	562877
					115	60	15184
					69.1	40	39604
AM2201 N-(2-fluoropentyl) isomer	C24H22FNO	FIU_0028	11.19	360.2	232.1	24	996175
					212.1	40	65149
					163.1	8	55708
					155	28	1542918
					144	44	343227
					127	56	4007228
					116	60	134909
					105	40	29289
					77.1	60	115888
					69.1	44	74493
AM694 4-iodo isomer	C20H19FINO	FIU_0032	11.23	436.1	232.2	28	35373

					230.9	28	1773251
					202.9	56	832575
					144	56	37593
					130.1	60	4162
					116	60	12023
					104	60	177754
					76.1	60	457749
					75	60	1955
					50.1	60	11667
BB-22 3-carboxyindole metabolite	C16H19NO2	FIU_0616	10.9	258.14158	214.1	12	4904
					176	16	4751
					169.4	12	81
					158.1	12	56
					132	16	6106
					118	24	9732
					97.1	20	3226
					91	52	3165
					69.1	28	1686
					55.1	36	15064

BB-22 7-hydroxyquinoline isomer	C25H24N2O2	FIU_0625	11.61	385.18378	240.2	20	391274
					144	44	140304
					138.8	28	54
					116	60	22713
					97.1	40	23426
					69.2	52	12572
					55.1	60	127830
	C24H28N2O2	FIU_0247	8.336	377.2	280.1	16	232019
					229.1	16	571388
					144	40	86872
					121	24	1420122
					112.1	24	2979710
					98.1	40	1866851
					93.1	48	141629
					91.1	60	1054701
					70.1	60	576499
					58.1	60	697418
CP 47,497-C6-homolog	C20H32O2	FIU_0566	14.52	305.24023	329.3	4	5428
					219.1	16	4943

					175.1	8	5884
					107	20	2547
					71.2	12	3268
					57.1	20	2973
D-Amphetamine	C9H13N	FIU_0296	6.37	136.1048	119.1	4	316370
					91.1	16	490294
					77.1	48	6194
					65.1	44	148115
					63.1	56	13406
					51.1	60	24279
HU-308	C27H42O3	FIU_0189	11.74	415.3	271.1	12	11983
					229.1	16	20287
					215.1	12	23499
					151	16	22820
					133.1	16	7398
					91.1	56	15306
					85.1	24	10761
					79.1	52	3868
					71.2	28	24245

					57.1	36	16073
JP104	C25H30N2O3	FIU_0192	10.878	407.2	390.2	4	38459
					212.1	8	33233
					197	24	278416
					171.1	32	119934
					169.1	48	35209
					153	52	151802
					141.1	60	61728
					95.1	16	13345
JWH 007	C25H25NO	FIU_0193	11.73	356.2	228.1	24	449889
					158	40	303826
					144	44	3043
					130	60	98680
					127	56	3494643
					103.1	60	27864
					101	60	13402
					77.1	60	83629
					70.5	48	268
JWH 015	C23H21NO	FIU_0195	11.172	328.2	200.1	20	777955

						158	40	420150
						155	24	2581326
						130	56	189386
						128.2	60	21653
						127	48	4510605
						103	60	83019
						101	60	52107
						77.1	60	318626
						51.1	60	13237
JWH 018 2'-naphthyl isomer	C24H23NO	FIU_0034	11.75	342.2	272.1	16		2685647
					214.1	24		328459
					158	36		7347
					155	28		2429736
					144	40		206154
					130.1	52		5848
					127	60		1943403
					127	60		1515840
					116	60		88552
					101	60		8898

					89.1	60	16793
					77.1	60	50271
JWH 018 N-(1-methylbutyl) isomer	C24H23NO	FIU_0044	11.49	342.2	272.1	20	9613
					217	28	187
					214.1	20	30114
					155.1	24	39037
					144.1	36	76873
					127	48	277031
					116	60	32010
					101	60	2203
					89.1	60	9212
					77.1	60	11114
JWH 019 N-(3-fluorohexyl) isomer	C25H24FNO	FIU_0490	11.34	374.18419	246.1	24	101003
					241.2	48	311
					235.3	32	115
					234	44	96
					155	28	639768
					144	44	38027

					128.9	60	600
					127	60	556165
					116.1	60	15061
					55.1	56	35903
JWH 073 2-methylnaphthyl analog	C24H23NO	FIU_0211	11.37	342.2	200.1	24	2120190
					158	36	48654
					144	40	1439879
					141	44	2964161
					130.1	48	36665
					116.1	60	560384
					115	60	1398875
					89.1	60	117601
					57.1	44	171175
JWH 073 2'-naphthyl-N-(1-methylpropyl) isomer	C23H21NO	FIU_0050	11.44	328.2	155	28	450081
					127	52	1987
JWH 073 N-(1-methylpropyl) isomer	C23H21NO	FIU_0053	11.24	328.2	144.1	32	755
					127	48	2336

JWH 080	C24H23NO2	FIU_0513	11.35	358.17288	200.1	24	509551
					185.1	24	1681297
					157.1	44	656393
					144	44	305867
					142	56	269199
					128.2	60	165048
					127.1	60	468608
					116	60	121793
					114	60	217204
					57.2	48	32943
JWH 081	C25H25NO2	FIU_0213	11.76	372.2	214.1	24	1254254
					185	28	4107559
					157	48	1494001
					144	44	762692
					142	60	674803
					129.2	56	59356
					128.1	60	348682
					127	60	970491
					116	60	222687

					114	60	391649
JWH 081 2-methoxynaphthyl	C25H25NO2	FIU_0055	11.32	372.2	214.1	24	151975
					185.1	20	5629250
					170	48	687030
					155	48	29741
					144	44	126069
					142	56	1906954
					129	44	326360
					127	60	773441
					116	60	58571
					114	60	790815
JWH 081 6-methoxynaphthyl	C25H25NO2	FIU_0058	11.6	372.2	214.1	24	1625473
					157	44	1782476
					144	44	808030
					142	60	1017151
					129.2	56	27994
					128	60	179017
					127	60	154957

					116	60	237373
					114	60	157876
JWH 081 N-pentanoic acid metabolite	C25H23NO4	FIU_0517	10.42	402.16271	244.1	24	46305
					185.1	28	424044
					157.1	48	135914
					144	40	46203
					142	60	55310
					128.1	60	27248
					127	60	80740
					114.1	60	18875
					83.1	44	11446
					55.1	56	28615
JWH 122 3-methylnaphthyl isomer	C25H25NO	FIU_0062	11.77	356.2	169.1	20	2175
					141.1	44	1524
JWH 176	C25H24	FIU_0527	11.12	325.1878	268.1	20	8116
					255.2	12	50942
					254	16	11872
					253.1	40	18508

					240.2	40	8304
					239.1	56	10220
					218.7	8	100
					141	36	12363
					117	24	3150
					115	60	6072
JWH 203 4-chlorophenyl isomer	C21H22ClNO	FIU_0069	11.47	340.2	124.9	28	2654
JWH 250 5-hydroxyindole metabolite	C22H25NO3	FIU_0544	10.63	352.18344	216.1	24	29318
					204.1	16	44666
					160	40	42613
					146	44	29781
					131	40	23001
					121	20	573349
					93.1	36	52252
					91.1	60	326959
					77.1	60	20797
					65.1	60	25626
JWH 309 5'-isomer	C30H27NO	FIU_0549	11.81	418.20926	290.1	24	83642

					220.1	44	40195
					192.1	56	5214
					165.1	60	15594
					155	24	1635180
					127	60	1138691
					77.1	60	7667
JWH 398 2-chloronaphthyl isomer	C24H22ClNO	FIU_0078	11.39	376.2	214.1	24	231019
					189	28	4982582
					161	56	3251333
					158	36	6216
					144	40	217358
					130	56	9879
					126	60	1124469
					116	60	83838
					106.1	24	9821
					89.1	60	13033
JWH 398 N-(5-hydroxypentyl) metabolite	C24H22ClNO2	FIU_0552	10.87	392.13391	236.9	16	104

					230.2	28	7004
					189	20	465236
					161	52	267650
					144.1	48	11857
					126.1	60	41737
					69.1	44	4461
Ketazolam	C20H17ClN2O3	FIU_0239	10.29	369.1	285.1	12	280998
					257	32	16837
					241	52	8974
					228	36	14853
					222.1	40	22339
					193.1	48	43350
					180	40	7040
					154	40	40360
					105	32	6998
					91.1	56	13109
MAM2201 N-(2-fluoropentyl) isomer	C25H24FNO	FIU_0637	11.33	374.18419	232.1	24	71184
					212.1	44	5095
					169.1	24	233041

					144	48	22486
					141.1	48	161427
					115.9	60	8297
					115.1	60	60736
					69.1	44	5184
MAM2201 N-(5-chloropentyl) analog	C25H24ClNO	FIU_0641	11.39	390.15464	248.1	24	43010
					212.1	40	2622
					169.1	28	174982
					144	48	25179
					141.1	56	115194
					116.1	60	7616
					115.1	60	33935
					69.1	40	8541
MDA 19	C21H23N3O2	FIU_0241	11.96	350.2	322.2	8	15488
					321.2	12	17301
					105	16	4002871
					77.1	60	2858803
					51.1	60	146489
Methoxetamine	C15H21NO2	FIU_0246	7.104	248.2	175.1	16	1256161

					159	24	205586
					121	28	1834341
					115	60	143861
					91.1	56	785339
					78.1	60	322780
					77.1	60	399226
					67.1	24	526201
					65.1	60	335234
Mitragynine	C23H30N2O4	FIU_0251	8.04	399.2	367.2	20	467367
					238.1	24	1026914
					226.1	24	1137626
					174.1	36	2745239
					159	56	1035371
					143.6	56	178496
					129	36	373236
					117	60	301729
					110.1	36	663958
					75.1	56	403343
PB-22 3-hydroxyquinoline isomer	C23H22N2O2	FIU_0598	11.57	359.16813	214.1	20	3192364

					158.1	36	40789
					144	44	1346827
					131.1	40	142
					130.1	56	29738
					116	60	427352
					89.1	60	51329
					71.2	40	22734
					55.1	60	11832
PB-22 N-(5-hydroxypentyl) metabolite	C23H22N2O3	FIU_0610	10.05	375.16304	230.1	8	857722
					197.8	56	89
					144	44	393476
					130.1	40	4500
					116	60	100226
					89.1	60	8081
					87.1	36	6747
					69.1	44	117910
					67.1	52	5102
					57.1	52	3751
Pentylone (hydrochloride)	C13H17NO3	FIU_0257	7.182	236.1	218.1	8	1222013

16						188.1	16	1722198
						175	20	737605
						160.1	24	353181
						159	32	238298
						135	24	346013
						131.1	40	605366
						86.1	16	491089
						77.1	60	258434
						65.1	60	373987
	(-)-CP 47,497	C21H34O2	FIU_0555	14.55	319.25588	301.3	4	209
						256.9	16	1614
						226.9	28	1377
						197	36	1552
						194.8	56	99
						167	52	1286
	(+)-CP 47,497	C21H34O2	FIU_0092	11.67	319.3	233.2	16	5304
						175.1	4	6556
						133.1	52	11635
						121.1	32	21203

					107.1	28	3166
					107	28	29911
					85.1	16	15021
					77.1	60	11302
					57.1	24	21782
(±)3-epi CP 47,497-C8-homolog	C22H36O2	FIU_0095	11.68	333.3	193.1	0	17115
					175.1	4	12820
					141.1	4	30894
					107.1	24	45118
					107.1	20	6758
					85.1	12	42672
					81.1	36	7221
					79.1	60	9103
					77.1	60	8900
					57.1	20	48600
(±)-CP 55,940	C24H40O3	FIU_0099	11.67	377.3	359.3	0	11115
					233.1	8	4667
					219.1	24	112
					215.3	12	4306

					175.1	4	181028
					149	20	1142
					121	16	5476
					106	56	264
					79.1	48	1847
					71.2	24	3674
					57.1	44	2241
(±)-epi CP 47,497	C21H34O2	FIU_0100	11.67	319.3	287	8	2296
					257.1	16	2093
					226.9	28	1874
					197	40	1993
					193.1	0	30928
					175.1	4	9216
					127.1	4	60202
					107	28	3488
					107	20	71808
					85.1	12	57086
					81.1	28	10222
					79.1	60	14131

					77.1	60	14460
					71.2	16	5251
					71.1	12	104540
					57.2	24	3800
					57.1	20	68024
(±)-UR-144 N-(4-hydroxypentyl) metabolite	C21H29NO2	FIU_0644	10.79	328.21983	310.3	16	6376
					230.2	20	6965
					144.1	36	10728
					130.1	52	2681
					125.1	20	202233
					97.1	32	24631
					83.1	32	10543
					69.1	40	26954
					57.2	48	28668
					55.1	48	56750
(S)-(+)-JWH 073 N-(3-hydroxybutyl) metabolite	C23H21NO2	FIU_0512	10.31	344.15723	284.1	24	47787
					216.1	24	74270

					158	32	55357
					155.1	24	1562802
					144	44	36078
					141	24	10495
					130.1	48	47487
					127	56	1188832
					77.1	60	35611
					55.2	52	17134
1-(p-Fluorophenyl) piperazine (HCl)	C10H13FN2	FIU_0105	11.65	181.1	179.1	16	82769
					138	20	832868
					136	28	175034
					110.1	32	62902
					109	40	106281
					96	44	102483
					95	48	142506
					91.1	36	106754
					83.1	52	112263
					75.1	60	235458
2,3-MDMA	C11H15NO2	FIU_0359	6.82	194.11028	135	16	490752

					133.1	16	127405
					105.1	24	276094
					103.1	40	62415
					79.1	36	149327
					77.1	48	322564
					65.1	52	21005
					58.2	16	21083
					51.1	60	175635
2C-D	C11H17NO2	FIU_0104	N/A	196.1	179.1	8	2288847
					164.1	16	913337
					149	28	465147
					119	20	169923
					117	24	160816
					115	32	124114
					103.1	44	59413
					91.1	40	462609
					77.1	56	359642
					65.1	60	181139
2-Ethylethcathinone (hydrochloride)	C13H19NO	FIU_0112	11.66	206.2	160.1	12	542020

					159.1	16	863271
					144.1	32	1027195
					132.1	20	219652
					131	24	167422
					130.1	44	210817
					128	44	117071
					91.1	60	129534
					77.1	60	269153
2-Fluoroisocathinone	C9H10FNO	FIU_0330	5.09	168.07464	135	24	4109
					113	40	65
					103.1	24	156217
					97.1	36	3077
					95	48	4765
					77.1	40	158993
					75.1	60	15704
					51.1	60	70381
2-Fluoromethamphetamine (hydrochloride)	C10H14FN	FIU_0116	11.66	168.1	137.1	8	750768
					115	40	9398

					109	16	2471668
					89.1	48	74142
					83.1	48	842629
					81.1	60	24256
					65.1	60	21162
					63.1	60	181237
					59.1	52	136619
					57.1	60	384207
2-Methoxyamphetamine	C10H15NO	FIU_0284	7.19	166.11536	149.1	4	544004
					121.1	16	593311
					115.1	40	19178
					93.1	20	45978
					91.1	28	368652
					78.2	48	52770
					77.1	36	47385
					65.2	48	180614
					63.2	60	19293
					51.2	60	26662
3-Methylmethcathinone (hydrochloride)	C11H15NO2	FIU_0134	11.65	194.1	177	0	2631

					59.1	12	2363
4-Ethylethcathinone (hydrochloride)	C13H19NO	FIU_0136	11.65	206.2	160.1	16	838944
					159.1	20	1315599
					144.1	32	1419213
					130	44	280770
					117	32	179624
					115	60	224469
					105.1	32	354534
					91.1	56	209305
					77.1	60	448597
4-Fluoroethcathinone (hydrochloride)	C11H14FNO	FIU_0140	11.63	196.1	178.1	12	2304538
					149.7	16	1223699
					149.6	20	966491
					148	36	780187
					135	28	526489
					123	24	369768
					115	32	171261
					103	36	442324

					77.1	52	563787
					75	60	224247
4-MTA	C10H15NS	FIU_0295	7.76	182.09252	165.1	4	473458
5-chloro AB-PINACA	C18H25ClN4O2	FIU_0702	10.34	365.1666	348.1	4	843149
					320.1	12	1045490
					249	24	783163
					213.1	36	355339
					193	40	33221
					171	48	43639
					145	48	393527
					117	60	52354
					90	60	58269
					69.1	48	138929
5-fluoro AB-PINACA	C18H25FN4O2	FIU_0704	9.93	349.19615	346.2	4	136707
					332.1	4	180571
					318.2	12	137353
					233	24	186686
					213	36	49839
					177	40	18735

					144.9	48	92353
					127	60	103
					117	60	13273
					90	60	9513
					69.1	40	26330
5-fluoro NNEI 2'-naphthyl isomer	C24H23FN2O	FIU_0438	10.9	375.17944	232.2	20	510890
					212.2	44	4725
					206.2	16	11018
					158.1	44	4467
					144.1	44	257722
					130	60	4555
					116.1	60	86151
					89.1	60	7960
					69.2	48	19567
					61.2	60	3191
5-methoxy MiPT	C15H22N2O	FIU_0156	6.49	247.2	174.1	16	1800810
					159	32	658412
					143	36	285289
					131.1	44	506709

					130	60	914787
					117.1	56	51664
					115	60	119428
					103	60	125276
					86.1	12	3771516
					77.1	60	87730
AB-PINACA	C18H26N4O2	FIU_0721	10.63	331.20558	314.1	4	96165
					286.1	12	105232
					215	28	128210
					144.9	48	67364
					117	60	8023
					90	60	11346
					89	60	5188
AB-PINACA N-(2-fluoropentyl) isomer	C18H25FN4O2	FIU_0722	10.26	349.19615	332.1	4	45912
					304.1	12	68529
					304.1	12	55983
					233	24	64244
					221.4	20	62
					145	48	24467

ADB-PINACA isomer 3	C19H28N4O2	FIU_0736	10.9	345.22123	344.2	4	153923
					328.2	4	239780
					316.1	12	150944
					300.2	12	223351
					215.1	24	330429
					145	48	178614
					117	60	19663
					90	60	24380
					71.1	44	2710
AKB48 N-(5-hydroxypentyl) metabolite	C23H31N3O2	FIU_0742	11.3	382.24163	135.1	24	355301
					107	56	30633
					93	60	47552
					91	60	7279
					81.1	60	14406
					79	60	41974
					77	60	7750
					69.1	60	4276
					67.1	60	16265

					55.1	60	6995
AM679	C20H20INO	FIU_0168	11.24	418.1	291.1	20	426471
					274.1	28	115007
					234.1	32	260697
					230.9	28	2973420
					214.1	32	173774
					202.9	52	1447955
					144	56	140632
					105.1	60	85309
					104	60	218400
					76.1	60	987934
BB-22 5-hydroxyquinoline isomer	C25H24N2O2	FIU_0621	11.69	385.18378	240.2	20	210609
					144	44	84635
					116.1	60	11958
					97.1	40	12978
					69.1	48	7467
					55.2	60	72538
BB-22 6-hydroxyquinoline isomer	C25H24N2O2	FIU_0623	11.65	385.18378	384.1	8	1882

					240.2	24	235990
					144	48	110900
					116	60	16835
					97.1	44	15144
					69.1	52	9363
					55.1	60	92413
Buphedrone (hydrochloride)	C11H15NO	FIU_0173	11.73	178.1	132.1	16	429829
					131	24	660390
					130.1	36	455022
					117	28	97297
					103	52	81371
					91	24	369543
					77.1	56	372343
					65.1	56	112158
					51.1	60	178280
Cannabidiolic Acid	C22H30O4	FIU_0466	11.39	359.21441	341.2	8	138494
					261.2	24	11869
					219.1	32	39872
					149	48	4109

					135.1	52	3856
					109.1	32	3523
					81.1	56	3798
					69.2	44	3921
					67.1	56	4007
					55.2	56	3881
Cannabidol	C21H30O2	FIU_0175	11.41	315.2	193.1	20	125293
					135.1	16	49031
					123	36	74797
					107.1	28	29702
					93.1	24	50704
					91.1	56	27451
					81.1	40	29657
					77.1	60	38893
					69.1	36	29620
					67.1	60	26974
DiPT	C16H24N2	FIU_0753	7.12	245.19395	144	20	309287
					127.8	48	9431
					127	44	36728

					117	40	59920
					114.1	12	284873
					102.1	12	27627
					91	60	39868
					77.1	60	21887
					72.1	28	50462
					65	60	5835
EAM2201	C26H26FNO	FIU_0634	11.32	388.19984	232.1	28	44179
					183.1	28	144992
					160.1	8	1887233
					155.1	44	43132
					153.9	60	18501
					144	44	23726
					132.1	16	1119025
					131.1	20	907831
					129.1	60	13014
					128	60	5573
					115.1	60	8554
					108.4	40	61

					77.1	60	5325
Ethcathinone (hydrochloride)	C11H15NO	FIU_0184	11.74	178.1	252.1	8	839218
					130	32	796041
					117	32	465294
					105.1	24	465129
					103	48	142508
					79.1	40	179079
					77.1	56	689864
					51.1	60	282425
FUB-144	C23H24FNO	FIU_0658	11.33	350.18419	332.2	20	12241
					252.1	20	18632
					208.1	40	4827
					125.1	24	89402
					109.1	48	110712
					97.1	32	14949
					83.1	60	14134
					69.1	36	10200
					57.2	48	16244
					55.1	44	33068

IMMA	C23H23ClN2O4	FIU_0191	10.632	427.1	397.6	8	56
					340	12	12250
					312.1	16	555767
					277.1	24	3331
					139	28	2351337
					111	60	1084533
					109.8	60	4498
					88.1	20	240661
					75.1	60	60717
					70.1	56	9986
Isopentedrone	C12H17NO	FIU_0336	7.44	192.13101	214.1	12	289064
					174.1	8	373755
					161.1	8	371548
					132.1	16	88082
					119.1	16	72124
					117.1	32	13888
					105.1	16	34044
					91.1	24	845878
					77.1	52	29178

					65.1	60	257167
JWH 011	C27H29NO	FIU_0194	12.019	384.2	286.1	20	249426
					256.1	20	178975
					158	36	870935
					155	28	4555479
					155	20	5487416
					130	60	257321
					128.2	60	17946
					127	60	3210086
					103.1	60	62054
					77.1	60	49903
					57.1	48	218834
JWH 018 2'-naphthyl-N-(1-ethylpropyl) isomer	C24H23NO	FIU_0033	11.61	342.2	272.1	20	117170
					214.1	20	332603
					155.1	24	4424907
					155	24	4123852
					144.1	36	1201002
					127	56	3278115
					116	60	537671

					101.1	60	14286
					89.1	60	125795
					77.1	60	86488
					71.2	36	13697
JWH 018 4-hydroxyindole metabolite	C24H23NO2	FIU_0476	11.47	358.17288	230.1	24	224326
					160.1	44	141597
					136.1	20	98
					132.1	56	23863
					127.1	60	534774
					119.1	44	163
					104.1	60	60611
					77.2	60	13686
JWH 018 6-methoxyindole analog	C25H25NO2	FIU_0198	11.57	372.2	244.1	24	263233
					174	40	132347
					159	56	29070
					155	24	5443132
					146	56	55362
					131	60	18367

					127	56	3835770
					119	60	34856
					77.1	60	79865
					73.1	24	2137
JWH 018 N-(1,1-dimethylpropyl) isomer	C24H23NO	FIU_0041	11.39	342.2	254.1	28	21268
					244.1	32	11555
					155.1	28	2726479
					144	32	1585954
					127	60	2405313
					116	60	574236
					89.1	60	121416
					77.1	60	40370
					71.1	32	76660
JWH 019 5-hydroxyindole metabolite	C25H25NO2	FIU_0488	11.12	372.18853	244.2	28	72139
					160.1	44	45091
					155	28	519271
					153.7	36	370
					132	60	16848

					127	56	437691
					77.1	60	6784
JWH 019 N-(4-fluorohexyl) isomer	C25H24FNO	FIU_0491	11.19	374.18419	354.2	20	101853
					284.1	24	41656
					246.1	24	231534
					226.1	32	41555
					155	28	1937388
					144	44	149535
					127	56	1647994
					116	60	62989
					61.1	56	13933
					55.1	52	71557
JWH 031 2'-isomer	C21H23NO	FIU_0498	11.79	306.17796	178.1	20	176562
					155	20	2752284
					155	16	1951093
					150.1	24	44705
					127	48	1152641
					108.1	32	42143
					94.1	36	80856

					80.1	36	47529
					77.1	60	80591
					66.1	60	45869
					55.1	36	21314
JWH 073 N-(1,1-dimethylethyl) isomer	C23H21NO	FIU_0052	11.23	328.2	272.1	16	1669
					155.1	20	2421
					155	24	2364
					144	32	1288
					126.9	56	1728
JWH 081 7-methoxynaphthyl	C25H25NO2	FIU_0059	11.34	372.2	185	20	4316320
					170	48	2352312
					155	48	43037
					142	60	196452
					141	44	91334
					129	44	200102
					128	60	176903
					127	60	512663
					115.1	60	45727

					114	60	366408
JWH 122 6-methylnaphthyl isomer	C25H25NO	FIU_0064	11.75	356.2	169.1	24	1376
					141.1	40	987
MAM2201	C25H24FNO	FIU_0240	11.262	374.2	232.1	24	1392578
					169	28	5181833
					144	44	955675
					141	52	3555141
					130	48	17112
					116.1	60	342788
					115	60	1394190
					91.1	60	27397
					89.1	60	51675
					69.1	40	67392
MAM2201 N-(3-fluoropentyl) isomer	C25H24FNO	FIU_0638	11.37	374.18419	240.5	12	54
					232.1	24	56398
					212.1	36	6760
					169.1	28	247286
					144	48	20148

					141.1	52	149963
					116	60	8178
					115.1	60	56336
					69.2	48	9073
Meconin	C10H10O4	FIU_0245	7.326	195.1	162	20	505497
					151	24	175878
					133.6	28	188824
					105	40	229156
					79.1	24	265361
					78.1	40	175613
					77.1	44	684716
					65.1	56	164058
					51.1	60	300277
Mephedrone metabolite ((±)-Pseudoephedrine stereochemistry)	C11H17NO	FIU_0339	6.97	180.13101	162.1	8	926545
					147.1	20	179253
					131.1	20	108754
					117.1	44	38440
					115.7	32	50051

					115.1	48	60206
					105.1	36	76106
					91.1	32	130880
					77.1	60	53499
					56.2	28	37194
methyl-1-(5-fluoropentyl)-1H-indole-3-Carboxylate	C15H18FNO2	FIU_0691	10.44	264.13216	232.1	16	182691
					212	12	14638
					144	28	80617
					132	20	35542
					130	40	49536
					117	44	35650
					116	48	33363
					89	60	26384
					77.1	60	15587
					69.1	24	10189
methyl-1-(cyclohexylmethyl)-1H-indole-3-Carboxylate	C17H21NO2	FIU_0692	11.54	272.15723	240.1	16	279836
					190	16	174692

						176	16	159668
						144	24	287596
						132	20	75326
						130	36	70111
						117	44	74110
						116	48	83945
						97.1	20	61919
						55.1	36	398673
	methyl-1-pentyl-1H-indole-3-Carboxylate	C15H19NO2	FIU_0693	11.16	246.14158	214.1	12	627235
						190	12	92756
						146.1	16	56859
						144	24	276000
						132	20	92899
						130	36	163052
						117	36	98496
						116	44	97392
						89	60	78764
						77.1	60	66247

Naphyrone (hydrochloride)	C19H23NO	FIU_0253	8.69	282.2	211.1	16	1840801
					155	28	927427
					141	24	3502131
					127	56	692483
					127	52	817886
					115	60	709417
					97.1	56	183118
					84.1	40	553901
					72.1	32	159245
					70.1	20	327611
					55.1	56	206401
PB-22 5-hydroxyquinoline isomer	C23H22N2O2	FIU_0602	11.35	359.16813	214.1	20	2104504
					191.2	28	111
					158	36	27308
					144	44	881828
					130.1	48	18855
					116	60	282413
					89.1	60	31523

					71.2	40	14579
					55.2	60	8309
PB-22 7-hydroxyquinoline isomer	C23H22N2O2	FIU_0606	11.26	359.16813	214.1	16	501342
					158	40	6355
					144	44	210202
					130	48	3891
					126.1	56	55
					116	60	69366
					89	60	7991
					71.1	36	3665
Phenazepam	C15H10BrClN2O	FIU_0258	9.936	349	242	28	45458
					208.9	32	32583
					207.2	40	21438
					206.1	40	91010
					184	36	83160
					179.1	56	84484
					130	52	22823
					125	48	16533
					105	52	55953

					104.1	60	56184
Pyrovalerone	C16H23NO	FIU_0261	7.962	246.2	175.1	16	1636949
					126.1	28	1157605
					119	28	957692
					105.1	24	3033329
					91.1	48	1247783
					84.1	36	496208
					77.1	60	387889
					72.1	20	228273
					70.1	16	230108
					65.1	60	561876
RCS-8 3-methoxy isomer	C25H29NO2	FIU_0087	11.77	376.2	254.1	28	2784967
					228.1	16	1197165
					158	40	479159
					144	48	1545250
					132.1	28	686096
					121.1	24	5055167
					91.1	60	1083878
					69.1	52	1397720

Appendix 2. LOD, LOQ, and precision and bias values for all compounds in Mix 4 at three different concentration levels

Compound Name	LOD (ng/mL)	LOQ (ng/mL)	Low (5 ppb)		Medium (20 ppb)		High (80 ppb)	
			% CV	% Bias	% CV	% Bias	% CV	% Bias
Methiopropamine	0.006	0.017	5.0	-0.6	7.0	-0.9	8.2	0.7
3,4'-methylenedioxy- α -pyrrolidinopropiophenone	0.003	0.010	4.4	-1.5	6.0	-3.2	7.2	1.3
3,4-MDMA	0.009	0.027	4.4	0.5	7.4	-2.7	7.0	1.2
2-methylethcathinone	0.010	0.029	5.7	-3.9	8.8	-3.7	10.7	-2.9
6-APB	0.005	0.016	6.2	1.4	6.1	1.7	6.5	1.4
5-methoxy- α -ethyltryptamine	0.000	0.001	5.5	7.9	6.9	2.9	7.6	-0.7
acetyl fentanyl	0.002	0.005	3.9	-3.3	6.0	-3.9	7.3	-1.0
4-methoxy PCP	0.005	0.015	7.2	6.1	4.3	2.3	7.1	-0.8
butyryl fentanyl	0.007	0.022	5.9	-3.1	8.1	-0.7	9.4	0.6
25T2 NBOMe	0.001	0.004	4.0	-1.7	6.0	-0.7	6.9	-0.2
PB-22-N-(4	0.023	0.069	3.3	-4.2	5.4	-1.1	7.0	0.9
AB-PINACA-pentanoic acid metabolite	0.020	0.060	8.7	-7.7	7.4	-5.9	7.9	2.5
25G-NBOMe	0.002	0.005	3.1	-7.3	5.7	-8.3	10.5	4.5
Delorazepam	0.000	0.001	3.0	-5.5	5.4	3.6	6.4	0.8
JWH 203 N - (5-hydroxypentyl) metabolite	0.003	0.009	5.0	-0.5	5.5	-0.1	8.2	2.0
JWH 018 6-hydroxyindole metabolite	0.035	0.106	34.7	-38.5	49.1	-47.8	33.3	-28.1
A-834735	0.006	0.017	5.3	-2.4	6.1	0.3	8.0	-1.0
XLR 12	0.001	0.002	7.3	-22.8	10.6	18.2	15.5	10.1
AM2201 N-(3-chloropentyl) isomer	0.001	0.002	4.4	-9.3	4.1	4.2	5.2	-0.2
BB-22 5-hydroxyisoqui	0.004	0.011	15.6	-29.6	8.8	3.2	6.1	-1.3
UR-144	0.004	0.011	8.6	-40.1	9.9	-4.3	9.6	-2.1

JWH 387	0.001	0.002	7.9	-44.4	7.5	-2.4	8.0	0.6
(+)-cann	0.003	0.010	12.1	-40.8	6.5	9.3	6.7	0.0
Boldenone Cypionate	0.003	0.010	7.7	-39.1	5.8	-9.0	6.6	9.9
CB-13	0.013	0.038	6.1	-49.7	19.5	-31.3	15.2	16.0

Appendix 3. LOD, LOQ. R² values and precision and bias values for all compounds in Mix 5 at three different concentration levels

Compound Name	LOD (ng/mL)	LOQ (ng/mL)	Low (5 ppb)		Medium (20 ppb)		High (80 ppb)	
			% CV	% Bias	% CV	% Bias	% CV	% Bias
Levamisole	0.014	0.042	11.2	1.1	7.3	-1.8	10.3	1.3
3-fluoromethcathinone	0.005	0.014	10.1	-24.0	4.7	-8.4	4.2	-13.3
Acetyl norfentanyl	0.006	0.018	12.8	-0.2	4.2	-4.9	2.5	0.3
2-methoxymethcathinone	0.010	0.030	10.3	-1.7	6.1	-4.9	13.3	-6.1
Pentedrone metabolite ((±)-Ephedrine stereochemistry)	0.005	0.014	10.7	-1.7	6.5	-6.3	12.5	-5.9
6-APDB	0.029	0.087	12.3	6.8	13.7	-10.0	1.8	2.3
4-methoxy-α-Pyrrolidinobutiophenone	0.015	0.044	8.2	-1.0	6.5	-4.9	11.5	-0.8
α-Ethylaminopentiophenone	0.019	0.057	8.4	-1.7	6.0	-7.4	6.7	-1.8
4-methyl-α-pyrrolidinobutiophenone	0.017	0.050	12.8	2.0	10.6	2.3	6.9	4.3
2C-I	0.011	0.033	11.1	6.8	7.2	-1.6	12.5	2.3
4-methyl-α-ethylaminopentiophenone	0.018	0.053	12.4	2.4	10.2	0.0	5.8	3.8
2C-E	0.019	0.058	11.3	10.8	6.6	4.7	8.2	1.8
4-fluoro PV8	0.025	0.075	7.6	-4.3	9.0	-10.6	5.7	-1.2
25I-NBOMe 4-methoxy isomer	0.007	0.022	10.9	2.2	5.1	-4.2	2.4	-0.4
4-fluoro PV9	0.019	0.058	7.3	-4.7	9.1	-10.7	28.6	-7.1
(±)-ORG 28611	0.032	0.097	10.8	2.5	4.7	-2.0	3.7	-0.3
ADB-PINACA N-(5-hydroxypentyl) metabolite	0.008	0.025	13.1	0.5	4.9	-4.4	3.5	0.4

JWH 193	0.006	0.018	10.7	1.5	7.4	0.4	4.2	-1.9
PB-22 N-(4-hydroxypentyl) metabolite	0.007	0.022	8.6	4.7	6.0	-1.8	1.2	0.0
5-fluoro ADBICA	0.012	0.036	12.7	2.5	9.2	-2.2	2.9	-0.6
PB-22 3-carboxyindole metabolite	0.007	0.022	9.9	1.1	7.7	-4.4	9.7	-1.0
5-fluoro PB-22 8-hydroxyisoquinoline isomer	0.006	0.017	9.7	4.1	6.4	-1.0	1.7	1.6
ADB-PINACA	0.007	0.020	14.1	3.0	10.0	-1.6	2.9	-0.8
JWH 210 5-hydroxyindole metabolite	0.020	0.060	12.0	8.7	5.5	-6.6	3.3	-0.5
BB-22 6-hydroxyisoquinoline isomer	0.008	0.024	11.2	10.1	5.0	-4.5	4.3	1.6
RCS-8 4-methoxy isomer	0.018	0.053	17.5	14.9	5.3	3.9	7.6	3.0
AKB48 N-(4-fluorobenzyl) analog	0.017	0.051	16.6	21.3	9.2	13.2	5.7	0.3
JWH 210	0.011	0.033	10.5	14.3	6.8	-2.0	5.5	3.4

Appendix 4. LOD, LOQ, and precision and bias values for all compounds in Mix 6 at three different concentration levels (LC-QqQ-MS)

Compound Name	LOD (ng/mL)	LOQ (ng/mL)	Low (5 ppb)		Medium (20 ppb)		High (80 ppb)	
			% CV	% Bias	% CV	% Bias	% CV	% Bias
Mescaline	0.146	0.441	32.0	7.8	14.7	6.0	17.0	11.1
a-Pyrrolidinobuthiothiophenone	0.053	0.159	14.3	-6.0	11.5	-12.6	14.3	1.8
3,4-EDMC	0.058	0.175	14.6	-6.4	10.8	-9.5	18.6	2.6
para-Methoxymethamphetamine	0.054	0.163	12.0	-8.4	18.8	-21.5	26.3	-13.2
2-methox Ketamine	0.040	0.122	11.7	-7.9	8.9	0.0	15.1	-5.4
3-Methoxyamphetamine	0.100	0.303	11.9	-20.8	5.1	-13.8	22.3	-18.5
2,5-DMMA	0.043	0.129	13.5	-3.4	8.9	-8.5	8.8	1.6
Pentedrone Metabolite ((+/-)-Psuedoephedrine stereochemistry)	0.038	0.114	11.3	-1.9	6.5	-5.6	14.0	-1.6
(+)-3,4-Methylenedioxy Pyrovalerone	0.035	0.105	7.3	-5.1	5.1	-5.4	8.7	2.2
3-Bromoamphetamine	0.041	0.124	11.8	-5.0	12.4	-6.4	20.8	-11.7
Propylhexdrine	0.040	0.121	11.6	-5.5	18.9	-14.0	22.4	-11.8

2C-TFM	0.037	0.114	8.8	-3.9	12.8	-9.9	14.8	-3.3
DOI	0.068	0.206	11.4	-5.8	9.5	-6.2	12.0	-3.0
2C-T-7	0.041	0.125	10.0	-2.7	9.2	-5.3	11.1	-0.6
Mepirapim	0.022	0.068	10.4	-2.5	9.9	-1.4	12.5	-6.3
MT-45	0.072	0.218	14.7	-0.4	13.2	14.6	19.3	7.3
AM1248 azepane isomer	0.014	0.044	8.5	-3.6	6.5	-6.0	8.3	0.5
1'-naphthoyl indole	0.051	0.153	11.4	-2.7	8.0	-5.8	11.1	-4.4
(+/-) JWH 073 N-(3-hydroxybutyl) metabolite	0.039	0.118	10.3	-1.1	8.0	-0.4	8.6	0.9
5-fluoro PB-22 7-hydroxyisoquinoline isomer	0.038	0.115	8.6	0.1	5.8	-2.8	9.8	1.8
(+/-) WIN 55,212	0.031	0.093	11.7	1.4	10.4	4.5	8.0	7.8
UR-144 N-(2-hydroxypentyl) metabolite	0.032	0.095	14.0	-0.4	6.2	-0.9	8.1	3.2
PB-22 7-hydroxyisoquinoline isomer	0.042	0.127	13.4	-2.5	4.4	-2.1	5.8	0.4
JWH 251 4-methylphenyl isomer	0.045	0.137	20.1	0.7	12.5	4.8	9.2	1.2

Appendix 5. LOD, LOQ, and precision and bias values for all compounds in Mix 7 at three different concentration levels (LC-QqQ-MS)

Compound Name	LOD (ng/mL)	LOQ (ng/mL)	Low (5 ppb)		Medium (20 ppb)		High (80 ppb)	
			% CV	% Bias	% CV	% Bias	% CV	% Bias
Mescaline	0.146	0.441	32.0	7.8	14.7	6.0	17.0	11.1
a-Pyrrolidinobutiothiophenone	0.053	0.159	14.3	-6.0	11.5	-12.6	14.3	1.8
3,4-EDMC	0.058	0.175	14.6	-6.4	10.8	-9.5	18.6	2.6
para-Methoxymethamphetamine	0.054	0.163	12.0	-8.4	18.8	-21.5	26.3	-13.2
2-methoxy Ketamine	0.040	0.122	11.7	-7.9	8.9	0.0	15.1	-5.4
3-Methoxyamphetamine	0.100	0.303	11.9	-20.8	5.1	-13.8	22.3	-18.5
2,5-DMMA	0.043	0.129	13.5	-3.4	8.9	-8.5	8.8	1.6
Pentedrone Metabolite ((+/-)-Pseudoephedrine stereochemistry)	0.038	0.114	11.3	-1.9	6.5	-5.6	14.0	-1.6

(+)-3,4-Methylenedioxy Pyrovalerone	0.035	0.105	7.3	-5.1	5.1	-5.4	8.7	2.2
3-Bromoamphetamine	0.041	0.124	11.8	-5.0	12.4	-6.4	20.8	-11.7
Propylhexdrine	0.040	0.121	11.6	-5.5	18.9	-14.0	22.4	-11.8
2C-TFM	0.037	0.114	8.8	-3.9	12.8	-9.9	14.8	-3.3
DOI	0.068	0.206	11.4	-5.8	9.5	-6.2	12.0	-3.0
2C-T-7	0.041	0.125	10.0	-2.7	9.2	-5.3	11.1	-0.6
Mepirapim	0.022	0.068	10.4	-2.5	9.9	-1.4	12.5	-6.3
MT-45	0.072	0.218	14.7	-0.4	13.2	14.6	19.3	7.3
AM1248 azepane isomer	0.014	0.044	8.5	-3.6	6.5	-6.0	8.3	0.5
1'-naphthoyl indole	0.051	0.153	11.4	-2.7	8.0	-5.8	11.1	-4.4
(+/-) JWH 073 N-(3-hydroxybutyl) metabolite	0.039	0.118	10.3	-1.1	8.0	-0.4	8.6	0.9
5-fluoro PB-22 7-hydroxyisoquinoline isomer	0.038	0.115	8.6	0.1	5.8	-2.8	9.8	1.8
(+/-) WIN 55,212	0.031	0.093	11.7	1.4	10.4	4.5	8.0	7.8
UR-144 N-(2-hydroxypentyl) metabolite	0.032	0.095	14.0	-0.4	6.2	-0.9	8.1	3.2
PB-22 7-hydroxyisoquinoline isomer	0.042	0.127	13.4	-2.5	4.4	-2.1	5.8	0.4
JWH 251 4-methylphenyl isomer	0.045	0.137	20.1	0.7	12.5	4.8	9.2	1.2

Appendix 6. LOD, LOQ, and precision and bias values for all compounds in Mix 7 at three different concentration levels (LC-QqQ-MS)

Compound Name	LOD (ng/mL)	LOQ (ng/mL)	Low (5 ppb)		Medium (20 ppb)		High (80 ppb)	
			% CV	% Bias	% CV	% Bias	% CV	% Bias
HMA	0.027	0.080	7.3	4.5	6.2	5.9	1.6	-1.3
MBZP	0.023	0.069	5.7	-2.9	6.0	-2.0	1.6	-1.2
Cathine	0.060	0.181	18.7	7.4	5.2	7.7	10.6	-5.6
N-methyl-2-AI	0.246	0.744	23.9	27.1	14.8	10.4	17.8	0.7
Methylenedioxy Provalerone Metabolite 2	0.109	0.332	3.2	-0.5	6.9	-0.1	2.7	-0.1
Mephedrone	0.065	0.197	7.8	-1.9	6.2	2.4	1.8	-2.7

4-Methoxyamphetamine	1.372	4.157	17.3	-7.7	8.1	-0.8	2.5	-1.9
2C-T	0.026	0.078	6.5	2.6	5.7	5.7	2.3	-1.2
3,4-Dimethylethcathinone	0.033	0.099	2.8	4.1	6.7	3.4	2.5	-1.4
3C-P	0.046	0.139	7.0	4.1	4.6	6.3	2.2	-0.8
2,3-Dichlorophenylpiperazine	0.297	0.900	4.6	-2.8	5.3	-0.9	1.4	-0.9
25H-NBOMe	0.016	0.049	5.9	1.9	5.1	4.8	1.4	-0.9
NRG-3	0.211	0.639	4.0	-1.5	6.3	3.4	4.5	-4.5
Diclofensine	0.056	0.170	54.7	31.8	19.0	9.1	5.6	-2.4
5-fluoro NNEI	0.107	0.324	4.9	-13.7	6.1	4.2	3.9	0.7
FUB-PB-22	0.071	0.215	4.2	-5.6	4.8	10.9	2.5	-0.3
AB-CHMINACA	0.110	0.334	9.0	-14.4	6.1	13.2	3.8	1.3
AKB48 N-pentanoic acid metabolite	0.047	0.143	6.0	-8.6	4.9	7.8	4.2	0.0
JWH 251 3-methylphenyl isomer	0.100	0.303	4.2	-1.7	6.4	3.2	3.2	-0.2
UR-144 N-(2-chloropentyl) analog	0.020	0.062	8.5	-30.2	8.1	12.9	6.0	-0.9
BB-22 4-hydroxyquinoline isomer	0.023	0.069	9.3	-46.7	14.6	-0.6	16.7	0.9
Delta 9 THC	0.425	1.289	10.9	-46.6	9.6	-9.4	5.2	4.5
JWH 018 2-hydroxyindole metabolite	0.083	0.251	20.5	-29.0	8.4	31.4	6.7	-0.7

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PUBLICATIONS AND PRESENTATIONS

Kimble, A., Foy, G. (2014) *Presumptive Blood Testing using X-Ray Fluorescence to Determine Iron Content*. American Chemical Society National Meeting, March 2014, Dallas, TX.

Kimble A., Arroyo, L, DeCaprio, A.P. (2015) *Triggered MRM Database for the Comprehensive Detection of Novel Psychoactive Substances by LC-QqQ-MS*. Society of Forensic Toxicologists, October 2015, Atlanta, GA.

Kimble A., Arroyo, L, DeCaprio, A.P. (2016) *Triggered MRM Database for the Comprehensive Detection of Novel Psychoactive Substances by LC-QqQ-MS*. 5th Annual IFRI Forensic Science Symposium, March 2016, Miami, FL.

Arroyo L., DeCaprio, A.P., Gluodenis, T., Broomes, A., Eckberg, M., Kimble, A. and Seither, J. (2016) *Comprehensive Analytical Tools for the Identification of Emerging Drugs of Abuse*. National Institute for Standards and Technology Trace Evidence Data Workshop, July 2016, Gaithersburg, MD.

Kimble A., Arroyo, L, DeCaprio, A.P. (2017) *Method Validation Using Multiple Compound Mixtures for Screening/Confirmation of 800+ Novel Psychoactive Substances (NPS) by Liquid Chromatography/Triple Quadrupole/Mass Spectrometry (LC/QqQ/MS)*. American Academy of Forensic Sciences, February 2017, New Orleans, LA.

Chenevert, J.M., Kimble A., DeCaprio A.P., (2018) *Assessment of Incorporation of Amphetamine and Diazepam into Human Head Hair for Preparation of Hair Material*. American Academy of Sciences, February 2018, Seattle, WA.

Kimble A., DeCaprio A.P. (2018) *QuEChERS Extraction of Novel Psychoactive Substances from Biological Matrices*. American Academy of Forensic Sciences, February 2018, Seattle, WA.

Kimble A., DeCaprio A.P. (2018) *Solid Phase Extraction of Novel Psychoactive Substances from Biological Matrices*. PittCon,, February 2018, Orlando, FL.

Kimble A., DeCaprio A.P. (2019) *Comparison of Multiple Extraction/Purification Methods for Novel Psychoactive Substances from Biological Matrices*. American Academy of Forensic Sciences, February 2019, Baltimore, MD.